EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 11, Revision 2 (FGE.11Rev2): Aliphatic dialcohols, diketones, and hydroxyketones from chemical groups 8 and 10

EFSA Publication

Link to article, DOI:
10.2903/j.efsa.2011.1170

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 11, Revision 2 (FGE.11Rev2):

Aliphatic dialcohols, diketones, and hydroxyketones from chemical groups 8 and 10[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

KEYWORDS

Flavourings, safety, alpha-diketones, ketals, hydroxyketones, diols.

SUMMARY

The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 12 flavouring substances in the Flavouring Group Evaluation 11, Revision 2 (FGE.11Rev2), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 12 flavouring substances belong to chemical group 10, Annex I of the Commission Regulation (EC) No 1565/2000.

The present flavouring group includes 12 candidate substances; nine alpha-diketones or their corresponding alcohols or ketals [FL-no: 02.133, 06.134, 07.071, 07.152, 07.167, 07.168, 07.238, 07.248 and 07.260], and three beta-diketones or their corresponding hydroxyketones (of which one is a tertiary alcohol) [FL-no: 07.097, 07.165 and 07.184] all belonging to chemical groups 8 and 10.

---

1 On request from the Commission, Question No EFSA-Q-2009-00563, adopted on 17 June 2009.

2 Panel members Arturo Anadon, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertle, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Fidel Toldra, Rosemary Waring, Detlef Wölfle. Correspondence: cef-unit@efsa.europa.eu

3 Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings for the preparation of this Opinion: Ulla Beckman Sundh, Vibe Beltoft, Wilfried Bursch, Angelo Carere, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerard Pascal, Iona Pratt, Gerrit Speijers, Harriet Wallin and EFSA's staff member Kim Rygaard Nielsen for the preparatory work on this scientific Opinion.

Suggested citation: EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 11, Revision 2 (FGE.11Rev2):
Aliphatic dialcohols, diketones, and hydroxyketones from chemical groups 8 and 10. EFSA Journal 2011; 9(2):1170. [52 pp.]. doi:10.2903/j.efsa.2011.1170. Available online: www.efsa.europa.eu/efsajournal.htm

One of the 12 candidate substances possesses four chiral centres [FL-no: 06.134] two possesses two chiral centres [FL-no: 02.133 and 07.168] and four substances possesses one chiral centre [FL-no: 07.097, 07.167, 07.184 and 07.238]. One of the substances [FL-no: 07.260] is a mixture of four isomers.

Five of the candidate substances are classified into structural class I, six are classified into structural class II and one is classified into structural class III.

Eight of the 12 candidate substances in the present group have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the "Maximised Survey-derived Daily Intake" (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a "modified Theoretical Added Maximum Daily Intake" (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, the 12 candidate substances have European daily *per capita* intakes ranging from 0.0012 to 15 microgram, which are below the thresholds of concern for structural class I, II and III (1800, 540 and 90 microgram/person/day, respectively).

The candidate substance 3-methyl-2,4-nonadione [FL-no: 07.184] contains a structural 2,4-dione element similar to pentan-2,4-dione. The only genotoxicity data available for this substance was a valid unpublished GLP study in *S. typhimurium* and *E. coli* which were both negative. Similar negative result was obtained for pentan-2,4-dione in a valid GLP study in Salmonella, however, positive genotoxicity results were obtained in other studies both *in vitro* and *in vivo*. Due to this anticipated structural alert for genotoxicity (the 2,4-dione structure) the Procedure was not applied for 3-methyl-2,4-nonadione [FL-no: 07.184] and accordingly additional data on genotoxicity are required. For the remaining candidate substances, genotoxicity data are only available for a limited number of substances, and the genotoxicity could not be assessed adequately. However, the genotoxicity data available on these remaining 11 candidate substances do not preclude evaluation using the Procedure.

Ten of the 11 flavouring substances evaluated through the Procedure are expected to be metabolised to innocuous products.

For the remaining candidate substance evaluated through the Procedure, diacetyl-trimer [FL-no: 06.134] the data available do not allow to anticipate hydrolysis to innocuous products. No No Observed Adverse Effect Level (NOAEL) exists for the substance or a structurally related substance to provide an adequate margin of safety under the conditions of intended use and accordingly additional data are required.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

It is considered that on the basis of the default MSDI approach the ten of the 11 candidate substances evaluated through the Procedure [FL-no: 02.133, 07.071, 07.097, 07.152, 07.165, 07.167, 07.168, 07.238, 07.248 and 07.260] would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI they ranged from 1600 to 3900 microgram/person/day for the five candidate substances from structural class I. For one of these candidate substances [FL-no: 02.133] the estimated intake is above the threshold of concern of 1800 microgram/person/day for structural class I. For the six candidate substances, which are assigned to structural class II, the estimated intake based on the mTAMDI range from 1500 to 5400 microgram/ person/day, which is above the threshold of concern for structural class II of 540 microgram/person/day. For the one candidate substance [FL-no: 07.168] from structural class III the mTAMDI value is 1600 microgram/person/day, which exceeds the threshold of concern for structural class III of 90 microgram/person/day. The four candidate substances [FL-no: 07.097, 07.165, 07.167, 07.238], which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products.

Thus, for seven of the 11 candidate substances evaluated through the Procedure [FL-no: 02.133, 06.134, 07.071, 07.152, 07.168, 07.248 and 07.260] the intakes, estimated on the basis of the mTAMDI exceed the threshold for the structural class, to which the flavouring substances have been assigned. Therefore, more reliable exposure data are required. On the basis of such additional data, the substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. The stereoisomeric compositions have not been specified for three of the substances [FL-no: 06.134, 07.184 and 07.260]. One of the substances [FL-no: 07.260] is a mixture of four isomers (three positional isomers, where one of these can exists as two stereoisomers) and the composition of mixture is not specified. Furthermore, for [FL-no: 07.097] the minimum assay is too low, so information on secondary components of [FL-no: 07.097] is missing.

Thus, the final evaluation of the materials of commerce cannot be performed for four substances [FL-no: 06.134, 07.097, 07.184 and 07.260], pending further information. For the candidate substance diacetyl-trimer [FL-no: 06.134] additional metabolism/toxicity data are required, and for 3-methyl-2,4-nonadione [FL-no: 07.184] data on genotoxicity are required before it can be evaluated through the Procedure.

The remaining eight substances [FL-no: 02.133, 07.071, 07.152, 07.165, 07.167, 07.168, 07.238 and 07.248] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

The FGE is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 and to take into account additional information that has been made available since the previous Opinion on this FGE.

The Revision also includes newly notified substances belonging to the chemical groups evaluated in this FGE.

After the completion of the evaluation programme the Union List of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996a).

HISTORY OF THE EVALUATION

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.11	9 December 2004	http://www.efsa.europa.eu/en/science/afc/afc_opinions/815.html	6
FGE.11Rev1	17 April 2007	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902220233.htm	7
FGE.11Rev2	17 June 2009		12

The present Revision of FGE.11, FGE.11Rev2, includes the assessment of five additional candidate substances [FL-no: 06.134, 07.097, 07.168, 07.184 and 07.248]. Genotoxicity data are available for [FL-no: 06.134 and 07.184]. Metabolism data are available for [FL-no: 06.134].

One flavouring substance, pentan-2,4-dione (former [FL-no: 07.191]) was deleted from the Register of flavouring substances as it is considered genotoxic *in vitro* and *in vivo*. Accordingly, its use as chemically defined flavouring substance is toxicologically not acceptable.

The genotoxic potential of the alpha-beta unsaturated ketone 2-hydroxypiperitone [FL-no: 07.168] was evaluated in FGE.213 with respect to genotoxic potential. In FGE.213, 3-ethyl-2-hydroxy-2-cyclopenten-1-one [FL-no: 07.057] was considered representative for nine structurally related substances in FGE.213, among those 2-hydroxypiperitone [FL-no: 07.168]. No carcinogenicity was observed for 3-ethyl-2-hydroxy-2-cyclopenten-1-one [FL-no: 07.057] in rats. The Panel concluded that the structural alert for genotoxicity is overruled for 3-ethyl-2-hydroxy-2-cyclopenten-1-one [FL-no: 07.057] as well as for the nine structurally related substances (EFSA, 2009x). The Panel therefore concluded that 2-hydroxypiperitone [FL-no: 07.168] could be evaluated through the Procedure in FGE.11Rev2.

Cyclic alpha,beta-diketones as 2-hydroxypiperitone [FL-no: 07.168] has been evaluated with other FGE.19 substances, as they are in equlibrium with enolic forms, which are alpha,beta-unsaturated ketones. However, in the case of the acyclic alpha,beta-diketones in this evaluation, the enolic form is not significant, consequently these substances were not taken to FGE.19.

Since the publication of FGE.11Rev1 and the Minutes from the 7th Plenary meeting in which the conclusion on the FGE.11Rev2 was summarised, information on stereoisomeric composition and a boiling point has been provided by EFFA on the following six substances: [FL-no: 02.133, 07.097, 07.167, 07.168, 07.238 and 07.260] (EFFA, 2010a).

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a Union List according to Commission Regulation (EC) No 1565/2000 (EC, 2000a). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 11, Revision 2

1.1. Description

The present Flavouring Group Evaluation 11, Revision 2 (FGE.11Rev2), using the Procedure as referred to in the Commission Regulation (EC)No 1565/2000 (the Procedure – shown in schematic form in Annex I), deals with nine alpha-diketones or their corresponding alcohols or ketal and one beta-diketone and two beta-hydroxyketones. These 12 flavouring substances (candidate substances) belong to chemical groups 8 and 10 of Annex I of Regulation (EC) No 1565/2000 (EC, 2000a).

One flavouring substance, pentan-2,4-dione (former [FL-no: 07.191]) was deleted from the Register of flavouring substances as it is considered genotoxic *in vitro* and *in vivo*.

The 12 candidate substances under consideration in the present evaluation are listed in Table 1, as well as their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structures and specifications. This group of candidate substances includes nine alpha-diketones or their corresponding alcohols or ketals [FL-no: 02.133, 06.134, 07.071, 07.152, 07.167, 07.168, 07.238, 07.248 and 07.260], and three beta-diketones or their corresponding alcohols (of which one is a tertiary alcohol) [FL-no: 07.097, 07.165 and 07.184].

The outcome of the safety evaluation is summarised in Table 2a.

The hydrolysis products anticipated for the candidate ketals are listed in Table 2b.

The 12 candidate substances are closely related structurally to 13 aliphatic acyclic alpha-diketones and related alpha-hydroxyketones (supporting substances) evaluated at the 51st meeting of the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) in the group “Aliphatic acyclic and alicyclic alpha-diketones and related alpha-hydroxyketones” (JECFA, 1999a). The names and structures of the 13 supporting substances are listed in Table 3, together with their evaluation status.

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, they may have different chemical properties resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number, etc.).

One of the 12 candidate substances possesses four chiral centres [FL-no: 06.134] two possess two chiral centres [FL-no: 02.133 and 07.168] and four substances possess one chiral centre [FL-no: 07.097, 07.167, 07.184 and 07.238]. The stereoisomeric compositions have not been specified for three of these substances [FL-no: 06.134, 07.184 and 07.260]. One of the substances [FL-no: 07.260] is a mixture of four isomers (three positional isomers, where one of these can exists as two stereoisomers) and the composition of mixture is not specified.

1.3. Natural Occurrence in Food

Eight of the 12 candidate substances have been reported to occur in fruits, fruit juice, vegetables, bread, cheese, fish, meat, peanuts, cocoa, wine, drinks, beer, tea, and coffee. Quantitative data on the natural occurrence in food have been reported for five of these substances (TNO, 2000; EFFA, 2004x; Flavour Industry, 2005b)).

These reports are:

- Butane-2,3-diol [FL-no: 02.133]: 0.006 mg/kg in fish (lean), up to 90 mg/kg in cheddar cheese, up to 2.3 mg/kg in raspberry, up to 850 mg/kg in vinegar, 1.9 mg/kg in sherry and up to 2900 mg/kg in various types of wine.
- 3,3-Diethoxybutan-2-one [FL-no: 07.152]: up to 0.1 mg/kg in cognac and weinbrand.
- 4-Hydroxy-4-methylpentan-2-one [FL-no: 07.165]: up to 0.07 mg/kg in roasted chicken.
- 2-Hydroxypiperitone [FL-no: 07.168]: 36 mg/kg in black currant (buds).
- Octan-2,3-dione [FL-no: 07.248]: 0.1 mg/kg in fish (lean), up to 0.2 mg/kg in turkey (roasted), up to 0.07 in chicken (roasted), up to 0.112 mg/kg in Guinea hen, up to 0.03 mg/kg in beef (grilled, roasted), up to 108 mg/kg in lamb and mutton fat (heated), 0.01 mg/kg in peanuts.

According to TNO four of the substances, diacetyl-trimer [FL-no: 06.134], 3-(hydroxymethyl)octan-2-one [FL-no: 07.097], 3-hydroxy-2-octanone [FL-no: 07.238] and 1- or 3-hydroxy-5-methyl-2- or 3-hexanone [FL-no: 07.260] have not been reported to occur naturally in any food items (TNO, 2000).

2. Specifications

Purity criteria for the 12 candidate substances have been provided by the Flavour Industry (EFFA, 2003e; EFFA, 2004x; EFFA, 2007l; EFFA, 2010a; Flavour Industry, 2005a; Flavour Industry, 2005b) (see Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), specification of secondary components is needed for [FL-no: 07.097] (see Table 1).

Information on stereoisomerism/composition of mixture is needed for three substances [FL-no: 06.134, 07.184, and 07.260] (see Section 1.2 and Table 1).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the "Maximised Survey-derived Daily Intake" (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999a).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999a).

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000a). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The intake estimation is based on the "Maximised Survey-derived Daily Intake" (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[4] (Eurostat, 1998). This is derived for candidate substances from

[4] EU figure 375 millions. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

In the present Flavouring Group Evaluation, Revision 2 (FGE.11Rev2) the total annual volume of production of the seven candidate substances for use as flavouring substances in Europe has been reported to be approximately 180 kg (EFFA, 2003f; EFFA, 2004x; EFFA, 2007l; Flavour Industry, 2005a; Flavour Industry, 2005b). 120 kg is accounted for by 3-(hydroxymethyl)octan-2-one [FL-no: 07.097] and 25 kg is accounted for by butane-2,3-diol [FL-no: 02.133], 19 kg is accounted for by 1- or 3-hydroxy-5-methyl-2- or 3-hexanone [FL-no: 07.260] and 10 kg is accounted for by diacetyl-trimer [FL-no: 06.134]. For the 13 supporting substances the total annual volume of production has been reported by JECFA to be approximately 38000 kg. Diacetyl [FL-no: 07.052] accounts for 18000 kg and 3-hydroxybutan-2-one [FL-no: 07.051] accounts for 19000 kg (JECFA, 2000a).

On the basis of the annual volumes of production reported for the 12 candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Table 2a). The estimated daily *per capita* intake of 3-(hydroxymethyl)octan-2-one [FL-no: 07.097], butane-2,3-diol [FL-no: 02.133], 1- or 3-hydroxy-5-methyl-2 or 3-hexanone [FL-no: 07.260] and diacetyl-trimer [FL-no: 06.134] from use as a flavouring substance is 15, 3.0 microgram, 2.3 microgram and 1.2 microgram, respectively. The daily *per capita* intakes for each of the remaining substances are less than 0.37 microgram (Table 2a).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the present evaluation of the 12 candidate substances, information on food categories and normal and maximum use levels[5,6,7] were submitted by the Flavour Industry (EFFA, 2003e; EFFA, 2003f; EFFA, 2004x; Flavour Industry, 2005a; Flavour Industry, 2005b; EFFA, 2007a; EFFA, 2007l).

The 12 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

According to the Flavour Industry, the normal use levels for the candidate substances are in the range of 1 – 20 mg/kg food, and the maximum use levels are in the range of 5 - 100 mg/kg (EFFA, 2003e; EFFA, 2003f; EFFA, 2004x; Flavour Industry, 2005a; Flavour Industry, 2005b; EFFA, 2007l) (See Table II.1.2, Appendix II).

The mTAMDI values for the five candidate substances from structural class I (see Section 5) range from 1600 to 3900 microgram/person/day. For the six candidate substances from structural class II the mTAMDI values range from 1500 to 5400 microgram/person/day. For the one candidate substance [FL-no: 07.168] from structural class III the mTAMDI value is 1600 microgram/person/day.

[5] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).
[6] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).
[7] The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All 12
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All 12 except [FL-no: 07.260]
03.0	Edible ices, including sherbet and sorbet	All 12
04.1	Processed fruits	All 12 except [FL-no: 06.134]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Only [FL-no: 07.260]
05.0	Confectionery	All 12
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All 12 except [FL-no: 07.260]
07.0	Bakery wares	All 12
08.0	Meat and meat products, including poultry and game	All 12 except [FL-no: 06.134, 07.260]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All 12 except [FL-no: 06.134, 07.260]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All 12 except [FL-no: 07.260]
13.0	Foodstuffs intended for particular nutritional uses	All 12 except [FL-no: 06.134, 07.260]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All 12
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All 12
15.0	Ready-to-eat savouries	All 12 except [FL-no: 07.260]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All 12 except [FL-no: 07.260]

4. Absorption, Distribution, Metabolism and Elimination

Ketals are expected to be readily hydrolysed after ingestion under the acidic conditions in the stomach to the corresponding alcohols and ketones.

In vitro studies conducted with diacetyl-trimer [FL-no: 06.134] showed 100 % disappearance within 12 hours in simulated gastric juice and 75 % disappearance in artificial saliva at pH 9 within 45 minutes (Guentert, 2004). However, no data on the identities of the hydrolysis products formed in these studies were provided. For the candidate substance diacetyl-trimer [FL-no: 06.134] (1,1'-(tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone) the hydrolysis products anticipated under the acidic conditions in the stomach are equimolar amounts of diacetyl and 3-hydroxy-3-methyl-hept-2,5,6-trione (Table 2b).

The candidate substances, which are alpha- and beta-diketones, ketal, hydroxyketones or diols, are expected to be absorbed from the gastrointestinal tract. The metabolic fate of acyclic aliphatic diketones depends primarily on the position of the carbonyl function and the chain length. Aliphatic acyclic diketones and alpha-hydroxyketones which contain a carbonyl function at the 2-position (i.e. a methyl ketone) may undergo alpha–hydroxylation and subsequent oxidation of the terminal methyl group to eventually yield corresponding ketocarboxylic acids. The ketoacids are intermediary metabolites (e.g. alpha-ketoacids), which may undergo oxidative decarboxylation to yield carbon dioxide and an aliphatic carboxylic acid. The acid may be completely metabolised in the fatty acid

pathway and citric acid cycle. Beta-keto-acids and derivatives readily undergo decarboxylation. Along with alpha-keto- and alpha-hydroxyacids, they yield breakdown products, which are incorporated into normal biochemical pathways

Alternatively, the methyl-substituted diketones may be successively reduced to the corresponding hydroxyketones and diols, which are excreted in the urine as glucuronic acid conjugates. This pathway is favoured at elevated *in vivo* concentrations, especially for longer chain length ketones. Alpha-hydroxyketones or their diol metabolites may be excreted as glucuronic acid conjugates. If the carbonyl function is located elsewhere on the chain or in a ring, reduction is the predominant detoxification pathway.

A more detailed discussion on the metabolism of these alpha-, beta-diketones, a related ketal, hydroxyketones and diols follows in Annex III.

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

In FGE.11, one of the six candidate substances, pentan-2,4-dione, was, based on the genotoxicity data available, considered genotoxic *in vitro* and *in vivo* and accordingly, the Procedure was not applied for this substance. The new candidate substance 3-methyl-2,4-nonadione [FL-no: 07.184] contains a structural 2,4-dione element similar to pentan-2,4-dione. The only genotoxicity data available for this substance are two valid un-published GLP studies in *Salmonella typhimurium* and *Escherichia coli* which were negative. A similar negative result was obtained for 2,4-pentandione in a valid GLP study in *S. typhimurium*, however, positive genotoxicity results were obtained in other studies both *in vitro* and *in vivo*. Due to these data the Procedure was not applied to 3-methyl-2,4-nonadione [FL-no: 07.184] and accordingly additional data on genotoxicity are required.

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the remaining 11 candidate substances from chemical groups 8 and 10 the Procedure was applied. The stepwise evaluations are summarised in Table 2a.

Step 1

Five of the candidate substances [FL-no: 02.133, 07.097, 07.165, 07.167 and 07.238], are classified into structural class I according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978). Five of the candidate substances are classified into structural class II [FL-no: 06.134, 07.071, 07.152, 07.248 and 07.260] and the remaining substance [FL-no: 07.168] is classified into structural class III.

Step 2

Ketals are expected to be readily hydrolysed after ingestion under the acid conditions in the stomach to the corresponding alcohols and ketones.

For the candidate substance diacetyl-trimer [FL-no: 06.134] (1,1'-(tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone), anticipated hydrolysis products under the acidic conditions in the stomach are equimolar amounts of diacetyl and 3-hydroxy-3-methyl-hept-2,5,6-trione. *In vitro* studies conducted with diacetyl-trimer [FL-no: 06.134] in simulated gastric juice showed 100 % disappearance within 12 hours. Similar studies conducted in artificial saliva showed 75 % disappearance of diacetyl-trimer at pH 9 within 45 minutes (Guentert, 2004). However, no data on the identities of the hydrolysis products formed in these studies were provided. Therefore, the Panel

did not consider the data sufficient to conclude that this substance is hydrolysed to innocuous products and the evaluation of this substance accordingly proceeds via the B-side of the Procedure.

At the estimated levels of intake, the remaining ten candidate substances would not be expected to saturate metabolic detoxification pathways. They are considered to be metabolised to innocuous products. The evaluation of these ten candidate substances, therefore, proceeds via the A-side of the Procedure scheme (Annex I).

Step A3

The estimated levels of intake for the five candidate substances classified into structural class I are in the range of 0.0012 - 15 microgram/*capita*/day, which are below the human intake threshold of concern for structural class I (1800 microgram/person/day). The intakes of the four class II candidate substances evaluated through the B-side of the Procedure are 0.0012 – 2.3 microgram/*capita*/day, which also are below the human intake threshold for that class (540 microgram/person/day). The intake of the one class III candidate substance [FL-no: 07.168] is 0.0012 microgram/*capita*/day, which is below the human intake threshold for that class (90 microgram/person/day) (Table 2a).

Based on the results of the safety evaluation sequence of the Procedure, these ten candidate substances proceeding via the A-side of the Procedure scheme do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on MSDI approach.

Step B3

The estimated level of intake for the candidate substance diacetyl-trimer [FL-no: 06.134] classified into structural class II is 1.2 microgram/*capita*/day, which is below the human intake threshold for structural class II (540 microgram/person/day).

Step B4

No NOAEL exists for diacetyl-trimer [FL-no: 06.134] or a structurally related substance to provide an adequate margin of safety under the conditions of intended use and accordingly additional data are required.

6. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach

The estimated intakes for the five candidate substances in structural class I based on the mTAMDI approach range from 1600 to 3900 microgram/person/day. For one of the substances the mTAMDI value is above the threshold of concern for structural class I of 1800 microgram/person/day. For comparison of the intake estimate based on the MSDI approach and mTAMDI approach see Table 6.1.

The estimated intakes for the six candidate substances assigned to structural class II based on the mTAMDI range from 1500 to 5400 microgram/person/day, which are above the threshold of concern for structural class II substances of 540 microgram/person/day. For comparison of the MSDI- and mTAMDI values, see Table 6.1.

The estimated intake for the one candidate substance 2-hydroxypiperitone [FL-no: 07.168] assigned to structural class III based on the mTAMDI is 1600 microgram/person/day, which is above the threshold of concern for structural class III substances of 90 microgram/person/day. For comparison of the MSDI and mTAMDI values see Table 6.1.

So, for eight of the 12 candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.133	Butane-2,3-diol	3.0	3900	Class I	1800
07.097	3-(Hydroxymethyl)octan-2-one	15	1600	Class I	1800
07.165	4-Hydroxy-4-methylpentan-2-one	0.085	1600	Class I	1800
07.167	4-Hydroxyhexan-3-one	0.0012	1600	Class I	1800
07.238	3-Hydroxy-2-octanone	0.0049	1600	Class I	1800
07.071	Octane-4,5-dione	0.0012	1600	Class II	540
07.152	3,3-Diethoxybutan-2-one	0.088	1600	Class II	540
07.248	Octan-2,3-dione	0.37	1600	Class II	540
07.260	1- or 3-Hydroxy-5-methyl-2- or 3-hexanone	2.3	1500	Class II	540
06.134	Diacetyl-trimer	1.2	5400	Class II	540
07.184	3-Methylnona-2,4-dione	0.12	1600	Class II	540
07.168	2-Hydroxypiperitone	0.0012	1600	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2003e; EFFA, 2003f; EFFA, 2004x; EFFA, 2007l; Flavour Industry, 2005a; Flavour Industry, 2005b) the estimated combined daily *per capita* intake as flavourings of the ten remaining candidate substances assigned to structural class I, II or III is 22 microgram. This combined intake does not exceed the thresholds of concern for substances belonging to structural class I of 1800 or II of 540 or III of 90 microgram/person/day, respectively.

The 12 candidate substances [FL-no: 02.133, 06.134, 07.071, 07.097, 07.152, 07.165, 07.167, 07.168, 07.184, 07.238, 07.248 and 07.260] are structurally related to 13 supporting substances, which all are alpha-diketones or precursors evaluated by the JEFCA at its 51st meeting. For 12 of these supporting substances European annual production volumes have been provided by Flavour Industry. The 12 supporting substances are all assigned to structural class II. The total estimated combined daily intake of the candidate and supporting substances (in Europe) is approximately 4600 microgram/*capita*, which would exceed the threshold of concern for structural class II (540 microgram/person/day).

However, based on the high capacity of enzymes in the metabolic pathways, it is anticipated that the combined intake of candidate substances (22 microgram/*capita*/day) and supporting substances (4600 microgram/*capita*/day) would be metabolised efficiently and would not saturate these metabolic pathways. Further, based on the data available, two supporting substances (diacetyl [FL-no: 07.052] 2200 microgram/*capita*/day and 3-hydroxybutan-2-one [FL-no: 07.051] 2300 microgram/*capita*/day) out of the total of 25 candidate and supporting substances provide 95 % of the contribution. These are present in the body as endogenous compounds (Kawano, 1959; Gabriel et al., 1972) and they would not be expected to give rise to perturbations outside the physiological range (JECFA, 1999a). Therefore, at the level of exposure, based on the MSDI approach, the total combined intake as flavouring substances of the candidate and supporting substances would not be expected to be of safety concern.

8. Toxicity

8.1. Acute Toxicity

Data are available for two candidate substances ([FL-no: 02.133 and 07.165]) and for pentan-2,4-dione and for six of the supporting substances [FL-no: 07.018, 07.051, 07.052, 07.060, 07.064 and 09.264] evaluated by JECFA (JECFA, 1999a).

Oral LD_{50} values in rats and mice are in the range from 600 to 9000 mg/kg body weight (bw).

The acute toxicity data are summarised in Annex IV, Table IV.1.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Data on oral subacute toxicity are available for one candidate substance ([FL-no: 07.165]), for the structurally related pentan-2,4-dione and for three supporting substances [FL-no: 07.051, 07.052 and 07.077] evaluated by the JECFA (JECFA, 1999a). There are no studies available on chronic toxicity and carcinogenicity for the candidate substances. No Observed Adverse Effect Levels (NOAELs) in the range of 10 - 100 mg/kg bw/day in rats (and rabbits) have been derived from subacute studies for one candidate substance [FL-no: 07.165] and for pentan-2,4-dione and in the range of 90-330 mg/kg bw/day from subchronic studies in rats for two supporting substances [FL-no: 07.051 and 07.052].

Repeated dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

There are no data available for candidate substances. For supporting substances there is one developmental toxicity study available for diacetyl [FL-no: 07.052] in which no adverse effects were observed at the applied dose levels up to 1600 mg/kg bw/day in hamsters, mice and rats.

Developmental/reproductive toxicity data summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

In vitro data are available for four candidate substances [FL-no: 02.133, 06.134, 07.165 and 07.184], for the structurally related pentan-2,4-dione and for five supporting substances [FL-no: 07.051, 07.052, 07.060, 07.018 and 07.077].

For one of the candidate substances, 4-hydroxy-4-methylpentan-2-one [FL-no: 07.165], *in vitro* studies have been reported with negative results obtained in bacterial gene mutation assays with and without metabolic activation as well as in a chromosomal aberration assay in rat liver cells *in vitro*. For a second candidate substance butane-2,3-diol [FL-no: 02.133], there is only one Ames test reported to be negative, but the validity of the study cannot be evaluated. No evidence of mutagenicity was reported in standard or modified Ames assays considered valid when 3-methyl-2,4-nonanedione [FL-no: 07.184] and diacetyl-trimer [FL-no: 06.134] were incubated with various strains of *S. typhimurium* or *E. coli* at concentrations up to 5000 µg/plate, with and without metabolic activation (Stien, 2005b; Sasaki, 2006).

For pentan-2,4-dione, both *in vitro* and *in vivo* studies are available. In the various *in vitro* studies reported (reverse mutation assays (Ames Tests), microbial DNA repair tests and tests on primary DNA damage, gene mutation and chromosomal aberrations) negative results were observed in one Ames Test with five tester strains of *S. typhimurium* with and without metabolic activation. Positive results were found in two Ames Tests with *S. typhimurium* strain TA104 with or without metabolic activation. Positive results were also observed in the chromosomal aberrations test in the absence of

metabolic activation. For the three tests on microbial DNA repair both positive and negative results have been reported. However, they followed unusual study protocols and experimental details are insufficiently reported. Thus, the results are of limited validity. In two *in vivo* micronucleus studies using intraperitoneal dosing, which were performed in compliance with GLP and in accordance with OECD guideline 474 in mice significant increases in micronucleated polychromatic erythrocytes were observed in peripheral blood as well as in bone marrow. Test concentrations used were high and close to the LD_{50} determined in the same test system, however, there was no decrease in the ratio of PCE/NCE, on the contrary an increase was reported. The results were clearly positive in both studies which are considered as valid. The same test protocol was used in an *in vivo* micronucleus study in rats. However, test concentrations had to be reduced due to excessive mortality. Under these conditions, negative results were observed in the micronucleus test in rats. There were no significant changes in the proportion of PCE. Therefore, the validity of the results of this study is limited.

Genotoxicity studies were also performed with five supporting substances:

For 3-hydroxybutan-2-one (acetoin) [FL-no: 07.051] there is only one valid negative Ames test while data from other *in vitro* studies (results of which were reported to be negative) cannot be considered as valid. Diacetyl [FL-no: 07.052] was found able to induce gene mutations in *S. typhimurium* TA100 and TA104. Diacetyl was reported to produce mutations in the TK +/- locus of L5178Y mouse lymphoma cells. However, the concentration required for a two-fold increase in mutations results in a 62 % growth reduction, rendering this effect questionable (Whittaker et al., 2008). In an unpublished GLP study on *in vivo* micronucleus formation in $B6C3F_1$ mice diacetyl was reported negative, however, since the PCE/NCE ratio was not reported it is not clear whether the test substance reached the target organ (NTP, 1994c). Hexan-3,4-dione [FL-no: 07.077] slightly induced gene mutations in bacteria. No genotoxic activity was observed in valid *in vitro* studies with pentan-2,3-dione [FL-no: 07.060] and hexan-2,3-dione [FL-no: 07.018] (see Table IV.4.).

Conclusion on genotoxicity:

There are mutagenicity data on two candidate substances ([FL-no: 02.133, 06.134, 07.165 and 07.184]) and for the structurally related pentan-2,4-dione in this flavouring group evaluation.

4-Hydroxy-4-methylpentan-2-one [FL-no: 07.165] was not mutagenic in various *in vitro* studies in bacteria and yeast and did not induce chromosomal aberrations in rat liver cells. For butane-2,3-diol [FL-no: 02.133] negative results were reported in an *in vitro* gene mutation study, of which, however, the validity cannot be evaluated. No evidence of mutagenicity was reported in Ames assays considered valid when 3-methyl-2,4-nonanedione [FL-no: 07.184] and diacetyl-trimer [FL-no: 06.134] were incubated with various strains of *S. typhimurium* or *E. coli*.

The structurally related pentan-2,4-dione is genotoxic *in vitro* and *in vivo*. In *in vitro* studies considered as valid, it induced chromosomal aberrations without metabolic activation and sister chromatid exchanges with and without metabolic activation while no gene mutations were observed in bacteria and mammalian cells. *In vivo*, pentan-2,4-dione induced micronuclei in mice peripheral blood and bone marrow. It should be noted that only pentan-2,4-dione exhibits the structural feature of a methylene group which is activated due to its position between two carbonyl groups.

Mutagenicity data are available for five of the 13 supporting substances, giving mainly negative results. There is indication that diacetyl [FL-no: 07.052] has a weak genotoxic activity *in vitro*. However, diacetyl is reported to be endogenous in humans and is reported to be rapidly reduced to acetoin and further to butan-2,3-diol, for which there are no indication of mutagenicity.

Overall, the genotoxicity data available on candidate and supporting substances do not preclude evaluation of the candidate substances in the present group using the Procedure. For 3-methyl-2,4-nonadione [Fl-no: 07.184], which contains a structural 2,4-dione element similar to pentan-2,4-dione no *in vivo* genotoxicity data were available.

Genotoxicity data are summarised in Annex IV, Table IV.4 and Table IV.5.

9. Conclusions

The present flavouring group includes 12 candidate substances; nine alpha-diketones or their corresponding alcohols or ketals [FL-no: 02.133, 06.134, 07.071, 07.152, 07.167, 07.168, 07.238, 07.248 and 07.260], and three beta-diketones or their corresponding beta-hydroxyketones (of which one is a tertiary alcohol) [FL-no: 07.165, 07.097 and 07.184] all belonging to chemical groups 8 and 10.

One of the 12 candidate substances possesses four chiral centres [FL-no: 06.134] two possesses two chiral centres [FL-no: 02.133 and 07.168] and four substances possesses one chiral centre [FL-no: 07.097, 07.167, 07.184 and 07.238]. One of the substances [FL-no: 07.260] is a mixture of four isomers.

Five of the candidate substances are classified in structural class I, and six are classified in structural class II and one is classified in structural class III.

Eight of the 12 candidate substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, the 12 candidate substances have European daily *per capita* intakes ranging from 0.0012 to 15 microgram, which are below the thresholds of concern for structural class I, II and III (1800, 540 and 90 microgram/person/day, respectively).

On the basis of the reported annual production in Europe (MSDI approach) the combined intakes of the candidate substances, assigned to structural class I, II or III, is 22 microgram. This combined intake does not exceed the threshold of concern for a substances belonging to structural class I of 1800 or II of 540 or class III of 90 microgram/person/day, respectively. The supporting substances are all assigned to structural class II. The total combined estimated levels of intake of candidate and supporting substances is approximately 4600 microgram/*capita*, which would exceed the threshold of concern for structural class II (540 microgram/person/day). However, based on information on efficient metabolism and on presence in the body as endogenous compounds, there are no safety concerns from the combined intakes of the candidate and supporting substances.

The candidate substance 3-methyl-2,4-nonadione [FL-no: 07.184] contains a structural 2,4-dione element similar to pentan-2,4-dione. The only genotoxicity data available for this substance was a valid unpublished GLP study in *S. typhimurium* and *E. coli* which were both negative. Similar negative result was obtained for pentan-2,4-dione in a valid GLP study in Salmonella, however, positive genotoxicity results were obtained in other studies both *in vitro* and *in vivo*. Due to this anticipated structural alert for genotoxicity (the 2,4-dione structure) the Procedure was not applied for 3-methyl-2,4-nonadione [FL-no: 07.184] and accordingly additional data on genotoxicity are required. For the remaining candidate substances, genotoxicity data are only available for a limited number of substances, and the genotoxicity could not be assessed adequately. However, the genotoxicity data available on these 11 remaining candidate substances do not preclude evaluation using the Procedure.

Ten of the 11 flavouring substances evaluated through the Procedure are expected to be metabolised to innocuous products.

For the remaining candidate substance evaluated through the Procedure, diacetyl-trimer [FL-no: 06.134], the data available do not allow to anticipate hydrolysis to innocuous products. No NOAEL exists for the substance or a structurally related substance to provide an adequate margin of safety under the conditions of intended use and accordingly additional data are required.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

It is considered that on the basis of the default MSDI approach the ten of the 11 candidate substances evaluated through the Procedure [FL-no: 02.133, 07.071, 07.097, 07.152, 07.165, 07.167, 07.168, 07.238, 07.248 and 07.260] would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI they ranged from 1600 to 3900 microgram/person/day for the five candidate substances from structural class I. For one of these candidate substances [FL-no: 02.133] the estimated intake is above the threshold of concern of 1800 microgram/person/day for structural class I. For the six candidate substances, which are assigned to structural class II, the estimated intake based on the mTAMDI range from 1500 to 5400 microgram/ person/day, which is above the threshold of concern for structural class II of 540 microgram/person/day. For the one candidate substance [FL-no: 07.168] from structural class III the mTAMDI value is 1600 microgram/person/day, which exceeds the threshold of concern for structural class III of 90 microgram/person/day. The four candidate substances [FL-no: 07.097, 07.165, 07.167, 07.238], which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products.

Thus, for seven of the 11 candidate substances evaluated through the Procedure [FL-no: 02.133, 06.134, 07.071, 07.152, 07.168, 07.248 and 07.260] the intakes, estimated on the basis of the mTAMDI exceed the threshold for the structural class, to which the flavouring substances have been assigned. Therefore, more reliable exposure data are required. On the basis of such additional data, the substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. The stereoisomeric compositions have not been specified for three of the substances [FL-no: 06.134, 07.184 and 07.260]. One of the substances [FL-no: 07.260] is a mixture of four isomers (three positional isomers, where one of these can exists as two stereoisomers) and the composition of mixture is not specified. Furthermore, for [FL-no: 07.097] the minimum assay is too low, so information on secondary components of [FL-no: 07.097] is missing.

Thus, the final evaluation of the materials of commerce cannot be performed for four substances [FL-no: 06.134, 07.097, 07.184 and 07.260], pending further information. For the candidate substance diacetyl-trimer [FL-no: 06.134] additional metabolism/toxicity data are required, and for 3-methyl-2,4-nonadione [FL-no: 07.184] data on genotoxicity are required before it can be evaluated through the Procedure.

The remaining eight substances [FL-no: 02.133, 07.071, 07.152, 07.165, 07.167, 07.168, 07.238 and 07.248] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 11, REVISION 2

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 11, Revision 2

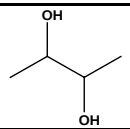
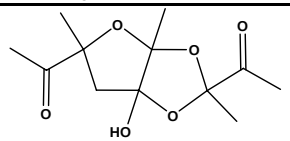
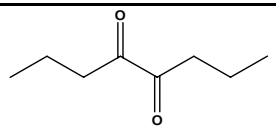
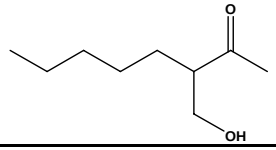
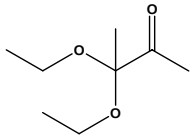
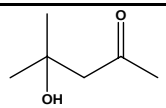
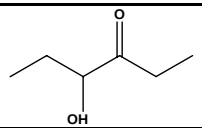
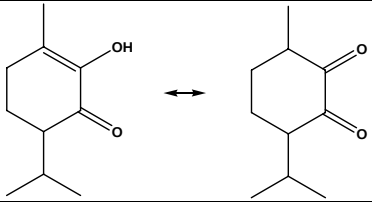
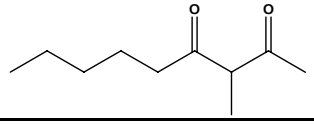
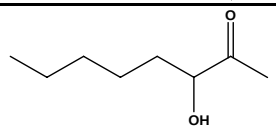
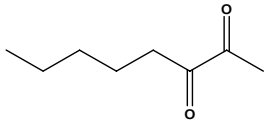
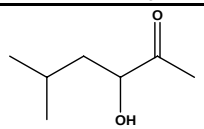
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.133	Butane-2,3-diol		10181 513-85-9	Liquid $C_4H_{10}O_2$ 90.12	Soluble 1 ml in 1 ml	181 MS 95 %	1.432-1.438 1.001-1.007	Racemate (EFFA, 2010a).
06.134	Diacetyl-trimer 6)		4303 18114-49-3	Solid $C_{12}H_{18}O_6$ 258.27	Soluble Soluble	 90 MS 95 %	n.a. n.a.	Stereoisomeric composition not specified by CASrn in Register. Register name to be changed to: 1,1'-(tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone.
07.071	Octane-4,5-dione		2141 5455-24-3	Liquid $C_8H_{14}O_2$ 142.20	Slightly soluble 1 ml in 1 ml	168 MS 95 %	1.415-1.421 0.907-0.913	
07.097 1839	3-(Hydroxymethyl)octan-2-one		3292 11113 59191-78-5	Liquid $C_9H_{18}O_2$ 158.24	Slightly soluble 1 ml in 1 ml	80 (0.3 hPa) NMR 92 %	1.416-1.422 0.874-0.878	Racemate (EFFA, 2010a). Min. Assay value 92 %, secondary components to be specified.
07.152	3,3-Diethoxybutan-2-one		 51933-13-2	Liquid $C_8H_{16}O_3$ 160.21	Slightly soluble 1 ml in 1 ml	164 MS 95 %	1.400-1.406 0.919-0.925	
07.165	4-Hydroxy-4-methylpentan-2-one		 123-42-2	Liquid $C_6H_{12}O_2$ 116.16	Slightly soluble 1 ml in 1 ml	165 MS 95 %	1.418-1.424 0.929-0.935	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 11, Revision 2

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
07.167	4-Hydroxyhexan-3-one		11108 4984-85-4	Liquid $C_6H_{12}O_2$ 116.16	Sparingly soluble 1 ml in 1 ml	167 MS 95 %	1.422-1.428 0.949-0.955	Racemate (EFFA, 2010a).
07.168	2-Hydroxypiperitone		4143 490-03-9	Solid $C_{10}H_{16}O_2$ 168.24	Slightly soluble 1 ml in 1 ml	233 82 NMR MS 98 %	n.a. n.a.	Racemate (EFFA, 2010a).
07.184	3-Methylnona-2,4-dione 6)		4057 113486-29-6	Liquid $C_{10}H_{18}O_2$ 170.25	Practically insoluble or insoluble 1 ml in 1 ml	52 (0.13 hPa) IR NMR MS 97 %	1.448-1.454 0.923-0.927	(R)- or (S)-enantiomer not specified by CASrn in Register.
07.238	3-Hydroxy-2-octanone		 37160-77-3	Liquid $C_8H_{16}O_2$ 144.21	Practically insoluble or insoluble 1 ml in 1 ml	91 (13 hPa) MS 95 %	1.431-1.437 0.927-0.933	Racemate (EFFA, 2010a).
07.248	Octan-2,3-dione		4060 585-25-1	Liquid $C_8H_{14}O_2$ 142.2	Soluble 1 ml in 1 ml	58 (1.3 hPa) IR NMR MS 95 %	1.419-1.424 0.905-0.913	
07.260	1- or 3-Hydroxy-5-methyl-2- or 3-hexanone 6)	 3-Hydroxy-5-methyl-2-hexanone shown	 163038-04-8	Liquid $C_7H_{14}O_2$ 130.18	Soluble Soluble	171-173 MS 95 %	0.921-0.933 1.419-1.431	Register name to be changed to 1-hydroxy-5-methyl-3-hexanone and 3-hydroxy-5-methyl-2-hexanone. Composition of stereoisomers and positional isomers not specified. CASrn in Register refers to the racemate of 3-hydroxy-5-methyl-2-hexanone.

1) Solubility in water, if not otherwise stated.

2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.
6) Stereoisomeric composition not specified.

TABLE 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

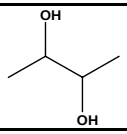
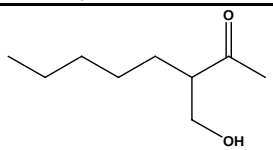
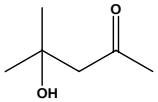
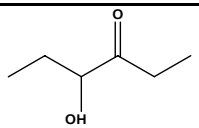
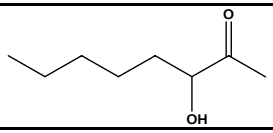
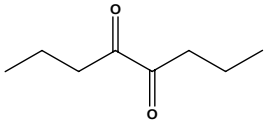
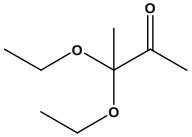
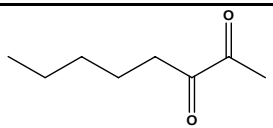
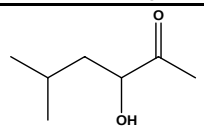
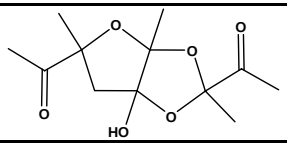
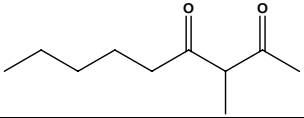
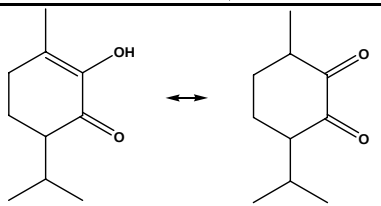
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.133	Butane-2,3-diol		3.0	Class I A3: Intake below threshold	4)	6)	
07.097 1839	3-(Hydroxymethyl)octan-2-one		15	Class I A3: Intake below threshold	4)	8)	
07.165	4-Hydroxy-4-methylpentan-2-one		0.085	Class I A3: Intake below threshold	4)	6)	
07.167	4-Hydroxyhexan-3-one		0.0012	Class I A3: Intake below threshold	4)	6)	
07.238	3-Hydroxy-2-octanone		0.0049	Class I A3: Intake below threshold	4)	6)	
07.071	Octane-4,5-dione		0.0012	Class II A3: Intake below threshold	4)	6)	
07.152	3,3-Diethoxybutan-2-one		0.088	Class II A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
07.248	Octan-2,3-dione		0.37	Class II A3: Intake below threshold	4)	6)	
07.260	1- or 3-Hydroxy-5-methyl-2- or 3-hexanone	 3-Hydroxy-5-methyl-2-hexanone shown	2.3	Class II A3: Intake below threshold	4)	7)	
06.134	Diacetyl-trimer		1.2	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
07.184	3-Methylnona-2,4-dione		0.12	Class II No evaluation			a)
07.168	2-Hydroxypiperitone		0.0012	Class III A3: Intake below threshold	4)	6)	

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot..

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

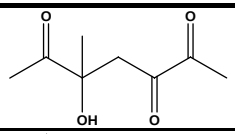
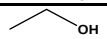
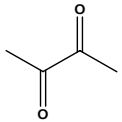
6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

a) Additional data on genotoxicity required

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE KETAL

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Ketal

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
-	3-Hydroxy-3-methyl-hept-2,5,6-trione		Not in register	Not in register	Anticipated hydrolysis products of diacetyl-trimer [FL-no: 06.134].
02.078	Ethanol 41		Category 1 a) No safety concern b)	No evaluation	At the forty-sixth JECFA meeting (JECFA, 1997a), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.
07.052	Diacetyl 408		No safety concern c) Category A d)	Class II A3: Intake above threshold, A4: Endogenous	

1) Category 1: Considered safe in use Category 2: Temporarily considered safe in use Category 3: Insufficient data to provide assurance of safety in use Category 4): Not acceptable due to evidence of toxicity.

2) No safety concern at estimated levels of intake.

3) Category A: Flavouring substance, which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.

4) Threshold of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.

5) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

a) (SCF, 1995).

b) (JECFA, 1997a).

c) (JECFA, 2000a).

d) (CoE, 1992).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

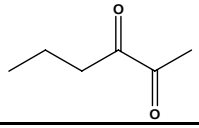
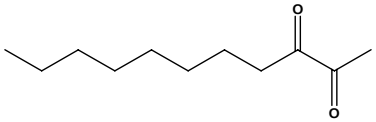
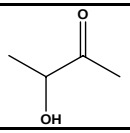
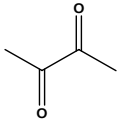
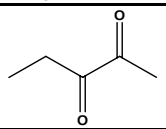
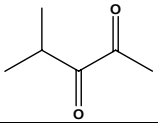
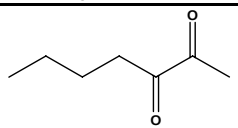
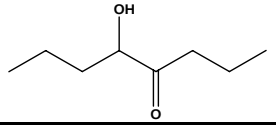
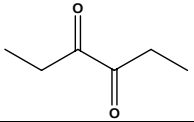
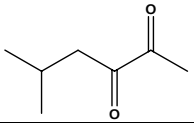
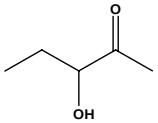
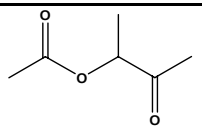
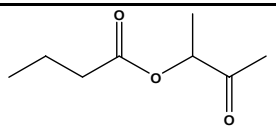
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
07.018	Hexan-2,3-dione		2558 152 3848-24-6	412 JECFA specification (JECFA, 2000d)	8.5	No safety concern a) Category A b)	
07.021	Undeca-2,3-dione		3090 155 7493-59-6	417 JECFA specification (JECFA, 2003b)	0.0037	No safety concern a) Category A b)	
07.051	3-Hydroxybutan-2-one		2008 749 513-86-0	405 JECFA specification (JECFA, 1998b)	2300	No safety concern a) Category A b)	JECFA evaluated acetoin (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
07.052	Diacetyl		2370 752 431-03-8	408 JECFA specification (JECFA, 1998b)	2200	No safety concern a) Category A b)	
07.060	Pentan-2,3-dione		2841 2039 600-14-6	410 JECFA specification (JECFA, 2003b)	130	No safety concern a) Category A b)	
07.063	4-Methylpentan-2,3-dione		2730 2043 7493-58-5	411 JECFA specification (JECFA, 2000d)	0.3	No safety concern a) Category A b)	
07.064	Heptan-2,3-dione		2543 2044 96-04-8	415 JECFA specification (JECFA, 1998b)	0.97	No safety concern a) Category A b)	

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
07.065	5-Hydroxyoctan-4-one		2587 2045 496-77-5	416 JECFA specification (JECFA, 2001c)	0.012	No safety concern a) Deleted b)	JECFA evaluated 5-hydroxy-4-octanone (CASrn as in Register). CASrn in Register refers to the racemate.
07.077	Hexan-3,4-dione		3168 2255 4437-51-8	413 JECFA specification (JECFA, 1998b)	21	No safety concern a) Category A b)	
07.093	5-Methylhexan-2,3-dione		3190 11148 13706-86-0	414 JECFA specification (JECFA, 2000d)	1.1	No safety concern a)	
07.125	3-Hydroxypentan-2-one		3550 11115 3142-66-3	409 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	JECFA evaluated 3-hydroxy-2-pentanone (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.186	sec-Butan-3-onyl acetate		3526 608 4906-24-5	406 JECFA specification (JECFA, 2000d)	0.024	No safety concern a) Category A b)	JECFA evaluated 2-acetoxy-3-butanone (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.264	sec-Butan-3-onyl butyrate		3332 10525 84642-61-5	407 JECFA specification (JECFA, 2000d)	0.012	No safety concern a)	JECFA evaluated butan-3-one-2-yl butanoate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.

1) EU MSDI: Amount added to food as flavouring substance in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

3) No safety concern at estimated levels of intake.

4) Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

a) (JECFA, 2000a).

b) (CoE, 1992).

ND) No intake data reported.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999a), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products[8] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[9] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

[8] "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).

[9] "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

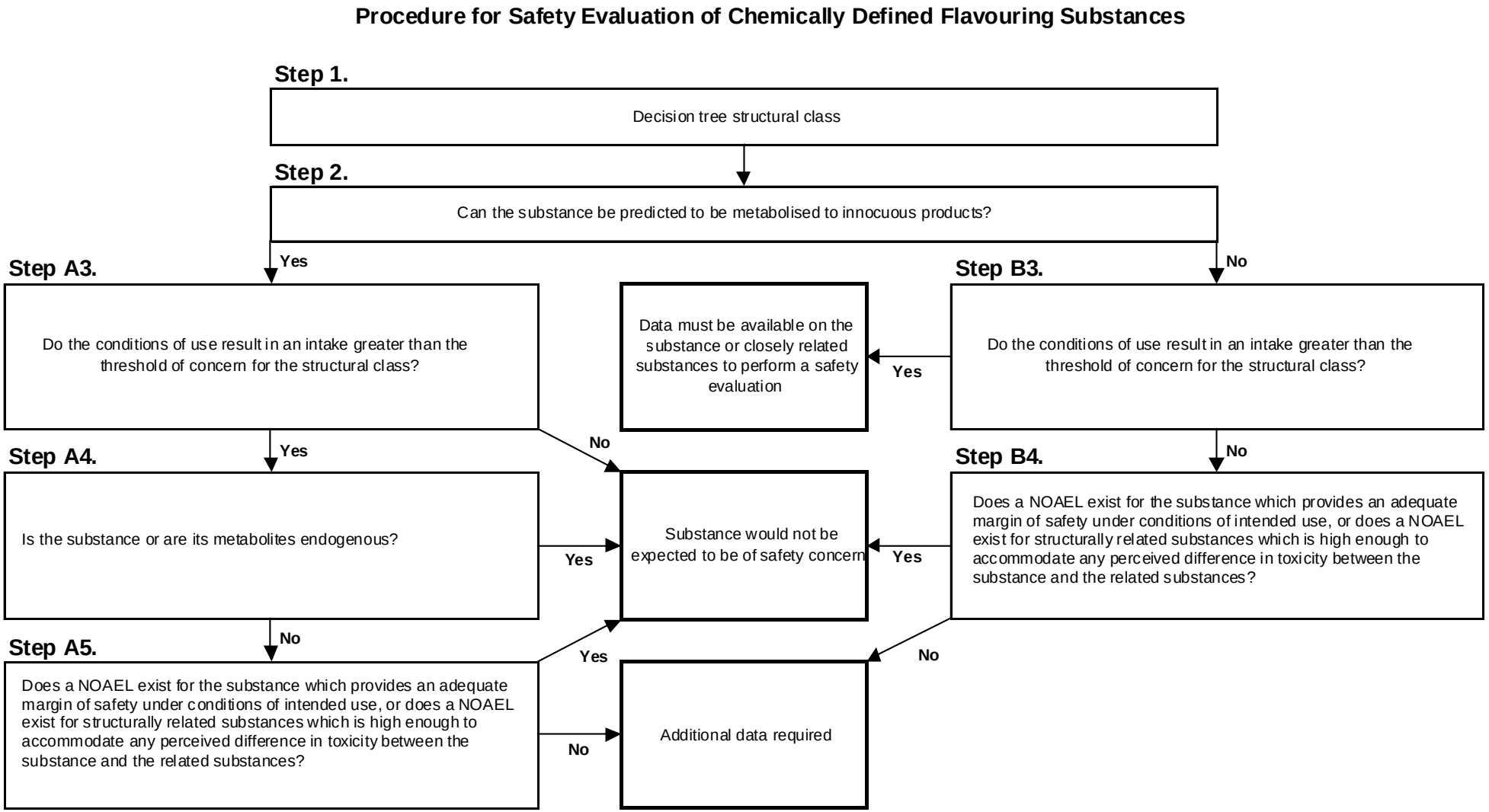


Figure I.I *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 2000a). According to the Industry the "normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000a)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The "normal and maximum use levels" are provided by Industry (EFFA, 2003e; EFFA, 2003f; EFFA, 2004x; EFFA, 2007a; EFFA, 2007l; Flavour Industry, 2005a; Flavour Industry, 2005b) for all the candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.11 (EFFA, 2003e; EFFA, 2003f; EFFA, 2004x; EFFA, 2007a; EFFA, 2007l; Flavour Industry, 2005a; Flavour Industry, 2005b).

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
02.133	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
06.134	20 100	10 50	5 10	- -	- -	10 50	20 50	20 50	- -	- -	- -	- -	20 100	- -	5 10	10 20	10 50	10 30
07.071	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.097	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.152	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.165	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.167	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.168	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.184	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.11 (EFFA, 2003e; EFFA, 2003f; EFFA, 2004x; EFFA, 2007a; EFFA, 2007l; Flavour Industry, 2005a; Flavour Industry, 2005b).

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
07.238	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
07.248	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
07.260	3	-	3	3	3	3	-	3	-	-	-	-	-	-	3	3	-	-
	6	-	7	6	6	6	-	3	-	-	-	-	-	-	6	6	-	-

II.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000a) and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (see Table II.2.3) are presented for all of the flavouring substances in the present flavouring group (EFFA, 2003e; EFFA, 2003f; EFFA, 2004x; EFFA, 2007l; Flavour Industry, 2005a; Flavour Industry, 2005b). The mTAMDI values are only given for highest reported normal use levels (see Table II.2.3).

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.133	Butane-2,3-diol	3900	Class I	1800
07.097	3-(Hydroxymethyl)octan-2-one	1600	Class I	1800
07.165	4-Hydroxy-4-methylpentan-2-one	1600	Class I	1800
07.167	4-Hydroxyhexan-3-one	1600	Class I	1800
07.238	3-Hydroxy-2-octanone	1600	Class I	1800
07.071	Octane-4,5-dione	1600	Class II	540
07.152	3,3-Diethoxybutan-2-one	1600	Class II	540
07.248	Octan-2,3-dione	1600	Class II	540
07.260	1- or 3-Hydroxy-5-methyl-2- or 3-hexanone	1500	Class II	540
06.134	Diacetyl-trimer	5400	Class II	540
07.184	3-Methylnona-2,4-dione	1600	Class II	540
07.168	2-Hydroxypiperitone	1600	Class III	90

ANNEX III: METABOLISM

III.1. Absorption, Distribution and Elimination

The candidate substances and supporting substances which are aliphatic acyclic alpha-diketones participate in a keto-enol equilibrium with the corresponding ketoenol (see Figure III.1). The keto form predominates (Gordon & Ford, 1972).

In rats and mice, orally administered acetoin (3-hydroxybutan-2-one [FL-no: 07.051]) is rapidly absorbed from the gastrointestinal tract (Gabriel et al., 1972). Upon injection of acetoin-2,3-^{14}C to albino rats, $^{14}CO_2$ appears in the expired air. The average 12-20 hours $^{14}CO_2$ production from acetoin-2,3-^{14}C was found to be 15 % after intraperitoneal (i.p.) administration (12 hours) and 47.7 % after intracardial administration (20 hours) (Gabriel et al., 1972).

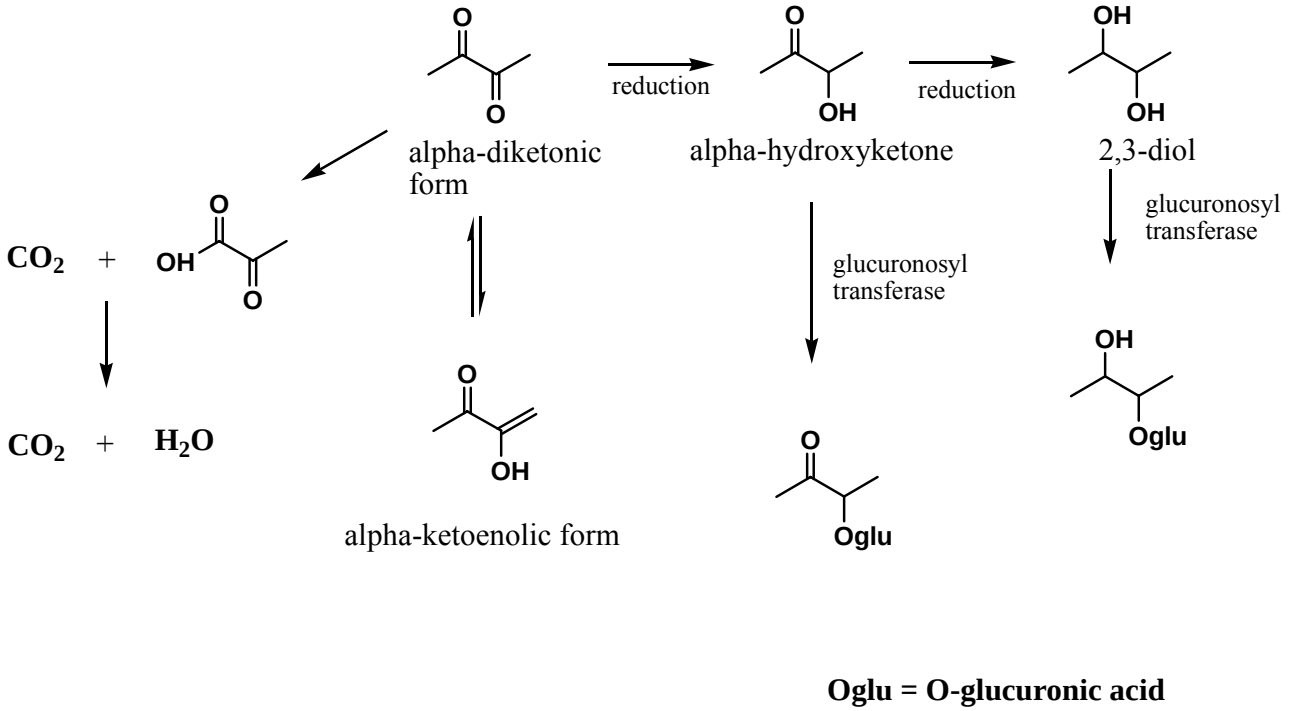


Figure III.1 *Keto-enol equilibrium and metabolism of aliphatic acyclic and alicyclic alpha-dicarbonyls*

III.2. Biotransformation

III.2.1. Hydrolysis

Ketals are anticipated to be readily hydrolysed after ingestion under the acid conditions in the stomach to their corresponding alcohol and ketone prior to absorption.

In vitro studies conducted on diacetyl-trimer [FL-no: 06.134] (1,1'-(tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone) in simulated gastric juice showed 100 % disappearance of diacetyl-trimer within 12 hours. Similar studies conducted in artificial saliva showed 75 %

disappearance of diacetyl-trimer at pH 9 within 45 minutes (Guentert, 2004). However, no data on the identities of the hydrolysis products formed were provided.

In general, esters are hydrolysed to their corresponding alcohol and carboxylic acid. Classes of enzymes recognized as carboxylesterases or esterases, the most important of which are the B-esterases, catalyse hydrolysis. Acetyl esters are the preferred substrates of C-esterases (Heymann, 1980). In mammals these enzymes occur in most tissues throughout the body (Anders, 1989; Heymann, 1980) but predominate in hepatocytes (Heymann, 1980). As an example, it is expected that the supporting chemicals, 2-acetoxy-3-butanone and butanon-3-one-2-yl butanoate, are metabolised in humans to acetic acid and butanoic acid, respectively, and acetoin.

III.2.2. Metabolism of Aliphatic Acyclic Diketones

The metabolic fate of acyclic aliphatic diketones depends primarily on the position of the carbonyl function and the chain length. Aliphatic acyclic diketones and alpha–hydroxyketones, which contain a carbonyl function at the 2-position (i.e. a methyl ketone) may undergo alpha–hydroxylation and subsequent oxidation of the terminal methyl group to eventually yield corresponding ketocarboxylic acids. The ketoacids are intermediary metabolites (e.g. alpha-ketoacids), which may undergo oxidative decarboxylation to yield carbon dioxide and an aliphatic carboxylic acid. The acid may be completely metabolised in the fatty acid pathway and citric acid cycle (see Figure III.1). Beta-keto-acids and derivatives readily undergo decarboxylation. Along with alpha-keto- and alpha-hydroxyacids, they yield breakdown products, which are incorporated into normal biochemical pathways (EFSA, 2005b).

Alternately, the methyl-substituted diketones may be successively reduced to the corresponding hydroxyketones and diols, which are excreted in the urine as glucuronic acid conjugates. This pathway is favoured at elevated *in vivo* concentrations, especially for longer chain length ketones. If the carbonyl function is located elsewhere on the chain, reduction is the predominant detoxification pathway.

Alpha-Hydroxyketones or their diol metabolites may be excreted as glucuronic acid conjugates (JECFA, 1999a).

Acetoin is metabolised primarily via oxidation at low concentrations *in vivo* and by reduction to 2,3-butanediol (butane-2,3-diol) at high concentrations. It is estimated that the rat liver is capable of oxidising 86 microgram (1 micromole) acetoin/g liver per day (Gabriel et al., 1972).

Oxidation of the terminal methyl group may form an alpha-ketoacid, which undergoes cleavage to yield CO_2 and a carboxylic acid fragment.

A total dose of 78 g of acetoin was administered to a dog over a two-month period. The doses were given orally in a 3 to 4 percent solution and subcutaneously in a 20 percent solution.

Urine was collected under toluene from the beginning of the dosing period through 40 hours after the last treatment. Butane-2,3-diol was identified as the major urinary excretion product, ranging from 5 to 25 percent of the dose. The remainder of the dose was completely metabolised (Westerfeld & Berg, 1943).

In liver preparations obtained from rats and rabbits, greater than 95 % of the radioactivity of 2,3-^{14}C-acetoin was detected as a mixture of stereoisomers of butane-2,3-diol (Gabriel et al., 1971). Although reductions of diacetyl and acetoin have been observed in animals *in vivo* and in animal tissue preparation *in vitro* at high concentrations, it appears that oxidation of diacetyl is a major endogenous metabolic pathway.

Reduction of ketones is mediated by alcohol dehydrogenase and NADPH dependent cytosolic carbonyl reductases (Bosron & Li, 1980). Reduction of acetoin and diacetyl is catalysed by the substrate-specific

enzymes diacetyl reductase and acetoin reductase, respectively. In rat liver mince, diacetyl, acetoin and butane-2,3-diol are interconvertible (Gabriel et al., 1972).

In male Wistar albino rats, a single oral dose of 5 mmol diacetyl/kg bw (430 mg diacetyl/kg bw) was metabolised by reduction to acetoin, which was present in high concentrations of major organs one hour after dosing. The subsequent reduction product, butane-2,3-diol, was detected in the liver, kidney and brain. Only 10 minutes incubation time was required to convert 10 nmol (9 x 10^{-4} mg) diacetyl to 3.7 nmol (3 x 10^{-4} mg) acetoin and 6.3 nmol (6 x 10^{-4} mg) butane-2,3-diol in rat liver homogenate (Otsuka et al., 1996). The organ-specific reductase activity was greatest in the liver and least in the brain (Otsuka et al., 1996).

Diacetyl and acetoin are reported to be endogenously formed in cats when pyruvate is converted to diacetyl and acetoin by pyruvate decarboxylase (Gabriel et al., 1972). Mean fasting blood acetoin levels of approximately 100 microgram acetoin/100 ml blood have been reported (Dawson & Hullin, 1954). Pyruvate also forms diacetyl *in vitro* in rat liver preparations (Järnefelt, 1955) and in microorganisms (Juni & Heym, 1956).

Diacetyl, acetoin and butane-2,3-diol are also reported to be endogenous in humans at levels of 0.25 – 0.75 microM, 2.2 microM and 5 -10 microM, respectively, in plasma. Plasma levels of diacetyl and acetoin, precursors of butane-2,3-diol were not affected by ethanol consumption, whereas plasma levels of butane-2,3-diol were elevated in individuals defective in aldehyde dehydrogenase (Otsuka et al., 1999) showing that acetoin is rapidly reduced to butan-2,3-diol in humans.

III.3. Studies on Candidate Substances

Butane-2,3-diol [FL-no: 02.133]:

Diacetyl, acetoin and butane 2,3-diol have been reported to be endogeous in humans. Higher levels of butane-2,3-diol, but not of diacetyl and acetoin were found in blood and urine of individuals defective in aldehyde dehydrogenase compared to normal individuals. This suggest that acetaldehyde formed from ethanol is converted to diacetyl, acetoin and eventually to butane-2,3-diol (Otsuka et al., 1999). The metabolic interrelationship of these chemicals is discussed above. Butane-2,3-diol may be an intermediate in the mammalian metabolism of acetaldehyde *in vitro*, and butane-2,3-diol and its oxidation metabolite, acetoin, have been reported as intermediates in the mammalian metabolism of pyruvate *in vitro* (Veech et al., 1987; Montgomery et al., 1993).

Butane-2,3-diol, 2-butanol and 3-hydroxy-2-butanone were identified as metabolites in the serum of guinea pigs injected i.p. with methyl ethyl ketone. 3-Hydroxy-2-butanone forms by alpha-hydroxylation of methyl ethyl ketone that subsequently forms butane-2,3-diol by reduction of the ketone function. The half-life of methyl ethyl ketone in serum was 270 minutes, and the clearance time of butane-2,3-diol was 16 hours (DiVincenzo et al., 1976). A proposed pathway of butane-2,3-diol elimination is as 2,3-butanediol beta-glucuronide after coupling with UDP-glucuronyltransferase (Otsuka et al., 1999).

4-Hydroxy-4-methylpentan-2-one [FL-no: 07.165]:

4-Hydroxy-4-methylpentan-2-one and 4-methyl-2-pentanol were detected in serum after i.p. injection of 4-methyl-2-pentanone to guinea pigs. 4-Hydroxy-4-methylpentan-2-one was the principal metabolite and was cleared in 16 hours. The concentration of 4-methyl-2-pentanol was too low for quantification. 4-Methyl-2-pentanone is metabolised by oxidation at the omega-1 carbon atom to form the hydroxylated ketone, 4-hydroxy-4-methylpentan-2-one, and to a lesser extent by reduction of the carbonyl group to form the secondary alcohol, 4-methyl-2-pentanol (DiVincenzo et al., 1976).

Pentan-2,4-dione:

Pentan-2,4-dione was investigated for its comparative pharmacokinetics in male F344 rats (4/1 group) by single intravenous (i.v.) injection of 4.3, 43, 148.5 and 430 mg/kg bw, or a 6-hour nose only inhalation exposure to ^{14}C-pentan-2,4-dione. Only the i.v. part of the study is reported here. The plasma concentration of ^{14}C-pentan-2,4-dione derived radioactivity declined in a biexponential fashion. The ^{14}C plasma concentration-time curves and derived pharmacokinetic parameters indicated that dose-linear kinetics occurred in the i.v. dose range of 4.3 to 148.5 mg/kg, but not at 430 mg/kg. Metabolism of pentan-2,4-dione was rapid in that the concentration of unmetabolised pentan-2,4-dione declined steadily to undetectable levels after eight hours. ^{14}C-pentan-2,4-dione derived radioactivity was eliminated mainly as exhaled $^{14}CO_2$ and in the urine. In the 48-hour samples for the 4.3, 43 and 148.5 mg/kg doses, $^{14}CO_2$ elimination was constant at 36.8, 38.8 and 42.3 %, and greater than the urinary ^{14}C excretion of 17.9, 14.3 and 29.6 %, respectively. However, at the 430 mg/kg i.v. dose there was a reversal of the excretion pattern, with the urine ^{14}C excretion (54.7 %) becoming greater than the exhaled $^{14}CO_2$ (27.3 %). Free parent compound and seven metabolites were detected in the 12-hour urine samples. In the 24-48-hour urine samples only one metabolite was detectable in small amounts (Frantz et al., 1998). At lower dose levels oxidation predominates, whereas at the high dose urinary hydroxylated metabolites formed by hydroxylation and ketone reduction predominate. These mechanisms are similar to those observed for diacetyl.

III.4. Conclusions on Metabolism

It is anticipated that humans will metabolise aliphatic acyclic methyl ketones principally by oxidation of the terminal methyl group at low levels of exposure. At higher levels, reduction to the diol and subsequent conjugation with glucuronic acid is a competing detoxification pathway. Other aliphatic acyclic diketones and hydroxyketones are reduced, conjugated with glucuronic acid and excreted. The ketals in the present FGE are anticipated to be hydrolysed to the corresponding alcohols and ketones. For one substance, diacetyl-trimer [FL-no: 06.134], the metabolism products can only partially be predicted.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for two candidate substances of the present flavouring group evaluation from chemical groups 8 and 10, for six supporting substances evaluated by the JECFA at the 51th meeting and for one structurally related compound (pentan-2,4-dione). The supporting substances are listed in brackets.

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(Acetoin [07.051])	Rat	NR	Oral	>5000	(Moreno, 1977d)	
Butane-2,3-diol [02.133]	Mouse	NR	Oral	9000	(Kopf et al., 1950)	
(Butan-3-one-2-yl butanoate [09.264])	Mouse	NR	Oral	>8000	(Pellmont, 1969a)	
	Rat	NR	Oral	>8000	(Pellmont, 1969a)	
(Diacetyl [07.052])	Rat	M, F	Gavage	M: 3400; F: 3000	(Colley et al., 1969)	
	Rat	NR	Gavage	1580	(Jenner et al., 1964)	
	Guinea pig	NR	Gavage	990	(Jenner et al., 1964)	
4-Hydroxy-4-methylpentan-2-one [07.165]	Rat	M	Gavage	4920	(Myers et al., 1977a)	
	Rat	M	Oral	4000	(Smyth, 1946a)	
(2,3-Pentanedione [07.060])	Rat	NR	Oral	3000	(Moreno, 1977d)	
[Pentan-2,4-dione]	Rat	M	Oral	1000	(Smyth, 1941)	
	Rat	M, F	Gavage	M: 780; F: 590	(Ballantyne et al., 1986) (Myers et al., 1985)	
	Rat	M	Oral	800	(Eastman Kodak Co., 1992a)	
	Mouse	M	Oral	951	(Eastman Kodak Co., 1992a)	
(2,3-Hexanedione [07.018])	Rat	NR	Oral	>5000	(Moreno, 1977d)	
(2,3-Heptanedione [07.064])	Rat	NR	Oral	>5000	(Moreno, 1979a)	

NR: Not reported

M= Male; F= Female

Subacute / subchronic / chronic / Carcinogenic toxicity data are available for one candidate substance of the present flavouring group evaluation from chemical groups 8 and 10, for three supporting substances evaluated by the JECFA at the 51th meeting and for one structurally related compound (pentan-2,4-dione). The supporting substances are listed in brackets.

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg/day)	Reference	Comments
(Acetoin [07.051])	Rat; M, F 30	Drinking water	0, 85, 330, 1345 mg/kg bw/d	90 Days	330	(Gaunt et al., 1972b)	Non-GLP study of good quality carried out as part of the BIBRA safety evaluation programme; published in a peer reviewed journal.
(Diacetyl [07.052])	Rat; M, F 30	Gavage	0, 10, 30, 90, 540 mg/kg bw/d	90 Days	90	(Colley et al., 1969)	Non-GLP study of good quality carried out as part of the BIBRA safety evaluation programme; published in a peer reviewed journal.
4-Hydroxy-4-methylpentan-2-one [07.165]	Rat; NR 10	Drinking water	0, 10, 40, 130 mg/kg bw/d	30 Days	10	(Smyth, 1946b)	Unpublished non-GLP study of poor quality with respect to study protocol. No histopathological examination of high dose and control group. No details available for method and results.
[Pentan-2,4-dione]	Rat; M 5	Gavage	0, 100, 500, 1000 mg/kg bw/d	1-15 Days[1]	100	(Eastman Kodak Co., 1992a)	Non-GLP study of 1979 in unpublished summary report. No details available for method and results. Quality of study limited with respect to study design. Results of the study have been published in Neurotoxicity of Industrial and Commercial Chemicals, Vol. 2, I.L. O'Donoghue, Editor, CRC Press, Boca Raton, Florida. p.77 (1985).
	Rat; M 5	Gavage	0, 100 mg/kg bw/d	14 Days[2]	100[3]	(Eastman Kodak Co., 1992a)	
	Rat; M 5	Gavage	0, 200 to 500 mg/kg bw/d	126 Days[4]	<200[5]	(Eastman Kodak Co., 1992a)	
	Rabbit; M 2	Gavage	0, 250, 500, 1000 mg/kg bw/d	14 Days[2]	250[6]	(Eastman Kodak Co., 1992a)	
(3,4-Hexanedione [07.077])	Rat; M, F 10-16	Diet	0, 17 mg/kg bw/d	90 Days	17[3]	(Posternak et al., 1969)	Summary of an unpublished non-GLP study (on 42 flavouring substances carried out in 1962-1967) prepared by BIBRA and published in a peer-reviewed journal.

M = Male; F = Female; NR= not reported.

[1] Animals dosed daily, between 1 and 11 times.

[2] Animals dosed ten times in fourteen days.

[3] The study was performed at a single dose level or multiple dose levels that produced no adverse effects.

[4] Animals dosed twice per day over a 126 day period at doses ranging from 100 to 250 mg/kg/day; animals that died or were killed during the study due to poor condition were replaced.

[5] NOAEL for the central nervous system was determined to be <200 mg/kg/day. The NOAEL for thymus toxicity was determined to be 200 mg/kg/day.

[6] One rabbit (50% of the group population) died due to possible aspiration of the test substance.

No developmental and reproductive toxicity data are available for candidate substances of the present flavouring group evaluation from chemical groups 8 and 10. Developmental and reproductive toxicity data are available for one supporting substance evaluated at the 51st JECFA meeting. Supporting substance is listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name [FL-no]	Study type Duration	Species/Sex No/Group	Route	Dose levels mg/kg bw/day	NOAEL (mg/kg/day), Including information of possible maternel toxicity	Reference	Comments
(Diacetyl [07.052])	Developmental toxicity: Gestation days 6-10	Hamster; F 21-25	Gavage	0, 16, 74.3, 345, 1600 mg/kg bw/d	1600 (maternal)[1, 3] 1600 (foetal)[2, 3]	(FDA, 1973)	Unpublished non-GLP study of limited quality with respect to possible developmental effects
	Developmental toxicity: Gestation days 6-15	Mouse; F 21-24	Gavage	0, 16, 74.3, 345, 1600 mg/kg bw/d	1600 (maternal)[1, 3] 1600 (foetal)[2, 3]	(FDA, 1973)	Unpublished non-GLP study of limited quality with respect to possible developmental effects
	Developmental toxicity: Gestation days 6-15	Rat; F 21-23	Gavage	0, 16, 74.3, 345, 1600 mg/kg bw/d	1600 (maternal)[1, 3] 1600 (foetal)[2, 3]	(FDA, 1973)	Unpublished non-GLP study of limited quality with respect to possible developmental effects

M = Male; F = Female.

[1] Based on observations of maternal survival, body weight and reproductive parameters.

[2] Based on observations of foetal survival and microscopic examination of foetal external, skeletal and soft tissues.

[3] The study was performed at a single dose level or multiple dose levels that produced no adverse effects and, therefore, a NOAEL was not determined. The NOAEL is probably higher than the reported dose level that produced no adverse effects.

In vitro mutagenicity/genotoxicity data are available for four candidate substances of the present flavouring group evaluation from chemical groups 8 and 10, for five supporting substances evaluated by the JECFA at the 51th meeting and for one structurally related compound (pentan-2,4-dione). Supporting substances are listed in brackets.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Acetoin [07.051])	Ames Test	*S. typhimurium* TA100	up to 4500 µg/plate	Negative[1]	(Garst et al., 1983)	Non-GLP study. Outcome of the study is only summarised with limited experimental details and no test results reported. Validity of the study cannot be evaluated.
	Ames Test	*S. typhimurium* TA100	390 µg/plate	Negative[2]	(Kim et al., 1987b)	Non-GLP study with limited information given on study protocol and results. Validity of the study cannot be evaluated.
	Ames Test	*S. typhimurium* TA98, TA100, TA102	0.44 – 44000 µg/plate	Negative[3]	(Aeschbacher et al., 1989)	Good quality, non-GLP study.
	Ames Test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 uvrA	1 – 5000 µg/plate	Negative[2]	(Iwata et al., 1984)	Published study in Japanese. Results (i.e. average number of revertant colonies per plate from three plates for each test concentration, including positive and negative controls) are given in table. Only tested without metabolic activation. Validity of the study cannot be evaluated.
Butane-2,3-diol [02.133]	Ames Test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 uvrA	1 – 5000 µg/plate	Negative[2]	(Iwata et al., 1984)	Published study in Japanese. Results (i.e. average number of revertant colonies per plate from three plates for each test concentration, including positive and negative controls) are given in table. Only tested without metabolic activation. Validity of the study cannot be evaluated.
(Diacetyl [07.052])	Ames Test	*S. typhimurium* TA100	90 µg/plate	Negative[3]	(Kim et al., 1987b)	Non-GLP study with limited information given on study protocol and results. Validity of the study cannot be evaluated.
	Modified Ames Test	*S. typhimurium* TA98, TA100, TA104; *E. coli* WP2 uvrA/pKM101	NR	Positive[1]	(Kato et al., 1989)	Only poorly reported abstract. Validity of the study cannot be evaluated.
	Modified Ames Test	*S. typhimurium* TA104	530 µg/plate	Positive	(Marnett et al., 1985a)	Published non-GLP study assessing the sensitivity of the new base substitution strains TA102 and TA104 to the mutagenic effects of carbonyls. Metabolic activation not reported. Due to the limited details reported on experimental design and results the validity of the study cannot be evaluated
	Ames Test	*S. typhimurium* TA104	5 – 500 µg/plate[4]	Positive[1] Negative[2]	(Shane et al., 1988)	Poorly reported non-GLP study of limited validity. Results are difficult to interpretate.
	Ames Test	*S. typhimurium* TA100, TA102	5 – 500 µg/plate[4]	Negative[3]	(Shane et al., 1988)	Poorly reported non-GLP study of limited validity. Results are difficult to interpretate.
	Ames Test	*S. typhimurium* TA100	152 – 950 µg/plate	Positive[2]	(Dorado et al., 1992)	Published non-GLP study of good quality. The number of revertants at the highest dose duplicated that of spontanous revertants. The effect was dose-related.
	Ames Test	*S. typhimurium* TA100	Approx. 400-600 µg/plate	Positive[3]	(Bjeldanes & Chew, 1979)	Published non-GLP study. Due to the limited details reported on experimental design and results the validity of the study cannot be evaluated.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames Test	*S. typhimurium* TA98	10 – 10000 µg/plate	Negative	(Bjeldanes & Chew, 1979)	Published non-GLP study. Due to the limited details reported on experimental design and results the validity of the study cannot be evaluated.
	Ames Test	*S. typhimurium* TA102	0.17 – 17200 µg/plate	Positive[3,]	(Aeschbacher et al., 1989)	Good quality, non-GLP study. The number of revertants at the highest dose duplicated that of spontanous revertants.
	Ames Test	*S. typhimurium* TA98, TA100	0.17 – 17200 µg/plate	Negative[3]	(Aeschbacher et al., 1989)	Good quality, non-GLP study.
	Modified Ames Test	*S. typhimurium* TA100	1.8 and 4 mM [4] (107 and 238 µg/pl)	Positive[2]	(Suwa et al., 1982)	Published non-GLP study with limited details reported on experimental design and results. The validity of the study is considered limited.
	Ames Test	*S. typhimurium* TA98, TA100, TA1535, TA1537,TA 1538; *E. coli* WP2 uvrA	1 – 5000 µg/plate	Negative[2]	(Iwata et al., 1984)	Published study in Japanese. Results (i.e. average number of revertant colonies per plate from three plates for each test concentration, including positive and negative controls) are given in table. Only tested without metabolic activation. Validity of the study cannot be evaluated.
	Ames Suspension Test	*S. typhimurium* TA1535, TA1537, TA1538	1%	Negative[3]	(FDA, 1974)	(not evaluated).
	Mutation	*S. cerevisiae*	NR	Negative[3]	(FDA, 1974)	(not evaluated).
	Chromosomal Malsegregation Assay[5]	*S. cerevisiae* D61.M	148 – 393 µg/ml	Negative	(Zimmermann & Mohr, 1992)	Published non-GLP study. Study is considered valid.
	Forward Mutation	Mouse lymphoma L5178Y TK+/- cells	100 – 250 µg/ml	Positive	(Whittaker et al., 2008)	Published non-GLP study. The result was positive. However, the concentration required for a two-fold increase in mutations result in a 62% growth reduction, rendering this effect questionable.
4-Hydroxy-4-methylpentan-2-one [07.165]	Ames Test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538;	100 – 10000 µg/plate	Negative[3]	(San & Klug, 1993)	Plate incorporation assay. Non-published GLP-study.
	Ames Test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2, WP2 uvrA	Up to 4000 µg/plate	Negative[3]	(Brooks et al., 1988)	Plate incorporation assay. Published study summarising an extended industry report. Study is considered valid.
	Mitotic Gene Conversion Assay	*S. cerevisiae*	10-5000 µg/ml	Negative[3]	(Brooks et al., 1988)	Published study summarising an extended industry report. Study is considered valid.
	Chromosome Aberrations	Rat liver epithelial type- cell line RL4	750, 1500, 2000, 3000, 4000 µg/ml	Negative	(Brooks et al., 1988)	Published study summarising an extended industry report. Study is considered valid.
(2,3-Pentanedione [07.060])	Ames Test	*S. typhimurium* TA100	105 µg/plate	Negative[2]	(Kim et al., 1987b)	Non-GLP study with limited information given on study protocol and results. Validity of the study cannot be evaluated.
	Ames Test	*S. typhimurium* TA98, TA100, TA102	0.9 – 90000 µg/plate	Negative[3]	(Aeschbacher et al., 1989)	Good quality, non-GLP study
[Pentan-2,4-dione]	Ames Test	*S. typhimurium* TA98, TA100, TA1535, 1537, 1538;	300 – 30000 µg/plate	Negative[3, 6]	(Guzzie & Morabit, 1985)	Valid unpublished GLP-study carried out according to US EPA test guidelines.
	Ames Test	*S. typhimurium* TA92, TA98, TA100, TA104	1.9 – 48 µmol/plate (190 –4805 µg/plate)[7]	Negative Positive [8]	(Gava et al., 1989)	No data on cytotoxicity reported. Metabolic activation not reported. Due to the limited experimental details reported the validity of the study cannot be evaluated.
	Modified Ames Test	*S. typhimurium* TA98, TA100, TA104; *E. coli* WP2 uvrA/pKM 101	NR	Negative[3] Positive[9] Positive[10]	(Kato et al., 1989)	Only abstract reported. Validity of the study cannot be evaluated.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Umu Test (DNA repair test)	*S. typhimurium* TA1535/ pSK1002	196, 410, 1235 µg/ml	Positive[3, 11,12]	(Ono et al., 1991)	Published non-GLP study. Unusual study design. Due to the limited experimental details and results reported the validity of the study cannot be evaluated. Relevance of results are questioned.
	Rec-Assay (DNA repair test)	*B. subtilis* H17 (Rec^+), M45 (Rec^-)	CR_{50} Rec^+ =209 µg/ml, $CR_{50}Rec^-$ = 195 µg/ml[13,2] CR_{50} Rec^+ =235 µg/ml, $CR_{50}Rec^-$ = 173 µg/ml[13,1]	Negative[2] Very weakly positive[1]	(Matsui et al., 1989)	Insufficient report of study design and experimental details. Detailed results not reported. Validity of the study cannot be evaluated.
	Mitotic aneuploidy (DNA repair test)	*S. cerevisiae* D61.M	0.74 – 1.96 % (7400 – 19600µg/ml)	Negative[2]	(Zimmermann et al., 1985a)	Published non-GLP study. Study is considered valid.
	Sister Chromatid Exchange	Chinese Hamster Ovary Cells	~0.01 – 1.0 µmol/ml (~1 – 100µg/ml)[14]	Positive	(Gava et al., 1989)	Published non-GLP study. Due to the limited experimental details and the incomplete cytotoxicity data reported the validity of the study cannot be evaluated.
	Sister Chromatid Exchange	Chinese Hamster Ovary Cells	20, 30, 100 µg/ml 30, 100, 300 µg/ml	Positive[2, 19] Positive[1, 19]	(Slesinski, 1986)	Unpublished GLP study. Study is considered valid**.**
	HGPRT Mutation Assay	Chinese Hamster Ovary Cells	10, 50, 100, 500, 1000 µg/ml	Negative[2, 15] Negative[1, 15]	(Slesinski, 1986)	Cytotoxic effects at 1 mg/ml. Unpublished GLP study. Study is considered valid**.**
	Chromosomal Aberrations	Chinese Hamster Ovary Cells	40 – 120 µg/ml (80, 100, 120 µg/ml) 60 – 140 µg/ml (100, 120, 140 µg/ml)	Positive[2,16, 20] Negative[1,16]	(Guzzie & Morabit, 1986)	Three highest conentrations analysed. Mitotic cell division not excessively reduced at conentrations used (i.e. not cytotoxic). Effects observed were chromatid breaks. Good quality unpublished GLP study.
(2,3-Hexanedione [07.018])	Chromosomal Malsegregation Assay[17]	*S. cerevisiae* D61.M	372 – 833 µg/ml	Negative[18]	(Zimmermann & Mohr, 1992)	Published non-GLP study. Study is considered valid.
(3,4-Hexanedione [07.077])	Ames Test	*S. typhimurium* TA100	228 – 4900 µg/plate	Very weakly positive[2]	(Dorado et al., 1992)	Published non-GLP study of good quality.
3-Methylnona-2,4-dione [07.184]	Revere mutation	*S. typhimurium* TA100, TA1535, TA98, TA1537	39, 78, 156, 313, 625 and 1250 µg/plate	Negative	(Sasaki, 2006)	Un-published GLP study. Study considered valid.
	Revere mutation	*E. Coli* WP2*uvrA*	39, 78, 156, 313, 625 and 1250 µg/plate	Negative	(Sasaki, 2006)	Un-published GLP study. Study considered valid.
Diacetyl-trimer [06.134]	Revere mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	100, 316, 1000, 3160 or 5000 µg/plate 10, 31.6, 100, 316 or 1000 µg/plate	Negative[3, 21,] Negative[22]	(Stien, 2005b)	Un-published GLP study. Study considered valid.

NR = Not reported.

CR50 = 50 % survival concentrations for the *B. subtilis* strains.

[1] With metabolic activation.

[2] Without metabolic activation.

[3] With and without metabolic activation.

[4] Estimated from graphical data.

[5] Pure chemical at 28°C, starting titer 14.6 x 10^6 cells/ml; and pure chemical, cold shock, starting titer 9.1 x 10^6 cells/ml.

[6] Test substance was cytotoxic at 30000 µg/plate.

[7] Calculated based on molecular weight = 100.12.

[8] Test substance was inactive towards TA92, TA98 and TA100 strains; however, it was mutagenic towards the TA104 strain.

[9] TA104 was positive in the absence of metabolic activation (specific activity = 2.25 revertants/µg); TA92, TA98 and TA100 were negative.

[10] WP2 uvrA/pKM 101 was positive in the presence of metabolic activation (specific activity = 7.73 revertants/µ g) and negative in the absence of metabolic activation.

[11] The highest concentration was only used in a 2-hour test (short term reaction) for which weakly positive result were reporteds.

[12] At 410 µg/ml a strongly positive results was reported after 24 hrs (long term reaction) with S9 metabolic activation, negative results were reported at 410 µg/ml after 2, 4 and 6 hrs with S9 metabolic activation and at 196 µg/ml after 2, 4 and 6 hrs and 20 hrs in the absence of S9 metabolic activation .

[13] The concentrations indicated are those of the test substance in the interaction period.

[14] Calculated based on molecular weight = 100.12.

[15] Cytotoxicity was observed, with and without S9 metabolic activation, at the 1.0 mg/ml dose level.

[16] Test substance was highly clastogenic in the absence of metabolic activation; however, in the presence of rat-liver S9 metabolic activation it was not clastogenic.

[17] Pure chemical at 28°C, starting titer 10.7 x 10^6 cells/ml; and pure chemical, cold shock, starting titer 11.3 x 10^6 cells/ml.

[18] In the pure form the test substance did not induce mitotic chromosome loss; however, almost all of the white colonies scored at the highest concentrations turned out to be respiratory deficient. The authors concluded that the test substance induces mitochondrial mutation under these experimental conditions.

[19] Pentan-2,4-dione-(purity 99.2%) was tested for sister chromatid exchange in Chinese hamster ovary (CHO) cells at culture concentrations of 6 to 100 micrograms/ml without metabolic activation and 10 to 300 micrograms/ml with metabolic activation. Experiments were carried out in duplicate. In preliminary experiments the appropriate range of test concentrations was determined for which the highest concentration would not kill more than 90% of the treated cells. Cytotoxic effects have been reported at concentrations of ≥1 mg/ml, concentrations above 2 mg/ml were lethal. Result: At the highest three doses evaluated for SCE (10, 30 and 100 micrograms/ml in the absence and 30, 100 and 300 in the presence of metabolic activation), pentan-2,4-dione produced significant ($p<0.001$) increases in the incidence of SCE in CHO cells both with and without metabolic activation. The SCE increase was greater without metabolic activation than with metabolic activation. A steep dose-response relationship was observed without S9, but not with S9. However, reproducible and statistically significant ($p<0.001$) increases were apparent in both tests. A remarkably high increase in the incidence of SCEs, which was higher than the positive control, was observed at 100 micrograms/ml without metabolic activation..Mitotic inhibition was evident only with the 300 micrograms/ml dose without S9.

[20] Pentan-2,4-dione (purity 99.2 %) was tested in a chromosomal aberration assay in Chinese Hamster Ovary Cells at concentrations of 40–120 micrograms/ml without metabolic activation and 60-140 micrograms/ml with metabolic activation. The three highest concentrations (80, 100 and 120 micrograms/ml without metabolic activation and 100, 120 and 140 micrograms/ml with metabolic activation) were analysed for chromosomal damage. Preliminary tests performed to assess effects on cell cycle division, indicated that pentan-2,4-dione produced a significant delay in cell division cycle, which was more pronounced in the absence than in the presence of S9. Test concentrations were selected on the basis of cytotoxicity data from preliminary experiments. Result: Statistically significant ($p>0.001$) increases in numbers of chromosome aberrations were observed at the three highest concentrations without S9 activation. However, in the presence of metabolic activation the cells tested did not demonstrate increased numbers of chromosome aberrations at any concentrations compared to control values.

[21] Standard plate-incorporation method, with and without S9.
[21] Modified pre-incubation method, with and without S9.

In vivo mutagenicity/genotoxicity data are available for one supporting substance evaluated by the JECFA at the 51th meeting and for one structurally related compound (pentan-2,4-dione). Supporting substance is listed in brackets.

TABLE IV.5: GENOTOXICITY (*IN VIVO*)

Chemical Name [FL-no]	Test system	Test Object	Route	Dose	Result	Refence	Comments
(Diacetyl [07.052])	*In vivo* Mouse Micronucleus Assay(bone marrow)	Mouse	Oral administration	300, 600, 1200, 2400 mg/kg; 300 mg/kg × 4 doses	Negative	(Iwata et al., 1984)	Published study in Japanese. Results (i.e. frequencies of PCEs and micronucleated PCEs, including positive and negative controls) are given in tables. No information can be found on sampling times. Validity of the study cannot be evaluated.
	In vivo Mouse Micronucleus Assay (bone marrow)	Mouse	Intraperitoneal injection	8, 16, 31, 62, 125, 250, 500 mg/kg	Negative	(NTP, 1994c)	Sampling at 24 hrs. Only summarised results of the study available. The PCE/NCE ratio was not reported so it is unclear whether the test substance has reached the bone marrow. Relevance of the results is limited.
[Pentan-2,4-dione]	*In vivo* Mouse Micronucleus Assay (peripheral blood)	Mouse	Intraperitoneal injection	200, 400, 650 mg/kg	Positive [2]	(Guzzie & Morabit, 1986)	Sampling at 30, 48, 72 hrs. Toxic effects during the study not reported (LD_{50} of 808 mg/kg). Unpublished valid GLP-study.
	In vivo Mouse Micronucleus Assay (bone marrow)	Mouse	Intraperitoneal injection	400, 650 mg/kg	Positive [3]	(Vergnes & Kubena, 1994a)	Sampling at 6, 24, 48 hrs. Toxic effects during the study not reported (LD_{50} of 808 mg/kg). Good quality GLP study carried out according to OECD and US EPA guidelines.
	In vivo Rat Micronucleus Assay (bone marrow)	Rat	Intraperitoneal injection	50, 100, 200 (400, 650) mg/kg[2]	Negative	(Vergnes & Kubena, 1994b)	Sampling at 6, 24, 48 hrs. Only summarised results of the study available. Unpublished valid study carried out according to EPA standards. Due to the lack of an effect on the PCE/NCE ratio it is unclear whether the test substance has reached the bone marrow. Relevance of the results is limited.

[1] Excessive mortality was observed at 400 and 650 mg/kg dose levels; therefore, these dose levels were replaced with 50 and 100 mg/kg.

[2] Pentan-2,4-dione (purity 99.2 %) was tested in an in vivo Mouse Micronucleus Assay. Swiss Webster Mice (5 animals per sex/dose group) were given i.p. injections of 200, 400 and 650 mg/kg. Peripheral blood was sampled at 30, 48 and 72 hours post injection. Results: In a dose-finding study using 579-1200 mg/kg toxicity was observed from 694-1200 mg/kg (20% to 100% mortality) and an LD_{50} of 808 mg/kg i.p. was found (95 % confidential interval 731.6-889.9 mg/ml). In this study, at 48 hrs post-injection, PCE/NCE ratio was reduced by 30 % and 23 % below the control levels for male and female animals that received a dose of 694 mg/kg, respectively.In the micronucleus study, the PCE/NCE ratio was determined in the 650 mg/kg group and in controls. No significant or dose-related decrease in the PCE/NCE ratio for either sex at any of the sample times (slight decrease with dose-related trend seen in females at 30hrs). In contrast, PCE/NCE ratio at 30 hrs was increased over control values in males at 400 and 650 mg/kg. A similar effect was not observed at any concentration at 48 hrs. A significant decrease in the PCE/NCE ratio (56.5% of the control) was observed with the positive control. The mean percentages of micronucleated PCEs were 0.38 and 0.22 for the vehicle control males and 0.12 and 0.14 for the vehicle control females sampled at 30 and 48 hrs, respectively. Mean percentages of micronucleated PCEs in CP-treated positive controls were 2.36 in males and 2.52 in females at 30 hrs post-treatment.At 30 hrs, a statistically significant increase in the incidence of micronucleated PCE was observed in the peripheral blood at 400 and 650 mg/kg. The effect was not dose-related. The mean percentages of micronucleated PCEs were 1.42 and 0.80 at 400 mg/kg and 1.16 and 0.80 at 650 mg/kg in males and females, respectively. At 48 hours a lower increase in micronucleated PCE than at 30 hrs was found at all concentrations tested. As there was no sex-related difference in the micronucleus response between males and females sampled at 48 and 72 hrs post-treatment, male and female values were combined for statistical analysis. A dose-related and statistically significant ($p<0.001$) increase in the incidence of micronuclei was observed at the 48 hrs sample period. A maximum incidence of 0.69 % (3.8 times the vehicle controls) micronucleated PCEs was observed for the highest dose level tested. The maximum ratio in the incidences of micronucleated PCEs compared to control was 6.7 (0.80 % at 400 mg/kg at 30 h in females compared to 0.12 % in control females). At 72 hrs the micronucleus response had returned to baseline levels.

[3] Pentan-2,4-dione (purity >98%) was tested in an *in vivo* Mouse Micronucleus Assay. Swiss Webster Mice (5 animals per sex/dose group) were given i.p injections of 400 and 650 mg/kg. Bone marrow was sampled 6, 24 and 48 hours post-injection.Results: Serious signs of toxicity were observed in both males and females at 650 mg/kg. Hypoactivity was seen in several males and females at 400 mg/kg. No serious signs of toxicity were observed in animals of either sex after day 1. In the micronucleus study, at 6 and 24 hrs a significant ($p<0.05$) increase in the PCE/NCE ratio over control values was observed at 400 mg/kg in males. No changes in the PCE/NCE ratio was seen in males at 48 hrs in either treatment group and in females of either treatment group at any sampling time. The mean percentages of micronucleated PCEs were 0.19, 0.29 and 0.18 for the vehicle control males and 0.20, 0.22 and 0.31 for the vehicle control females sampled at 6, 24 and 48 hrs, respectively. At 24 hrs the incidence of micronucleated PCEs was significantly increased in males and females at 400 and 650 mg/kg. The effect was not dose-related. The mean percentages of micronucleated PCEs were 0.81 and 0.97 at 400 mg/kg and 1.32 and 0.80 at 650 mg/kg in males and females, respectively. Mean percentages of micronucleated PCEs in CP-treated positive controls at 24 hrs post-treatment were 1.47 and 1.63% in males and females, respectively. The frequency of micronucleated PCEs was significantly increased at 24 hrs at 400 mg/kg and at 650 mg/kg, both in males and females. A maximum incidence of 1.32 % (4.6 times the vehicle controls) micronucleated PCEs was observed for the highest dose level

tested. This was also the maximum ratio in the incidences of micronucleated PCEs compared to control (1.32 % at 650 mg/kg at 24 h in males compared to 0.29 % in control males). No significant increase of micronucleated PCEs was observed at 6 and 48 hrs at 400 mg/kg and at 650 mg/kg in either sex.

REFERENCES

Aeschbacher, H.U., Wolleb, U., Loliger, J., Spadone, J.C., Liardon, R., 1989. Contribution of coffee aroma constituents to the mutagenicity of coffee. Food Chem. Toxicol. 27(4), 227-232.

Anders, M.W., 1989. Biotransformation and bioactivation of xenobiotics by the kidney. In: Hutson, D.H., Caldwell, J., Paulson, G.D. (Eds.). Intermediary xenobiotic metabolism in animals. Taylor and Francis, New York, pp. 81-97.

Ballantyne, B., Dodd, D.E., Myers, R.C., Nachreiner, D.J., 1986. The acute toxicity and primary irritancy of 2,4-pentanedione. Drug Chem. Toxicol. 9(2), 133-146.

Bjeldanes, L.F., Chew, H., 1979. Mutagenicity of 1,2-dicarbonyl compounds: maltol, kojic acid, diacetyl and related substances. Mutat. Res. 67, 367-371.

Bosron, W.F., Li, T.K., 1980. Alcohol dehydrogenase. In: Jakoby, W.B. (Ed.). Enzymatic Basis of Detoxification vol. 1. Academic Press, New York, 231-248.

Brooks, T.M., Meyer, A.L., Hutson, D.H., 1988. The genetic toxicology of some hydrocarbon and oxygenated solvents. Mutagenesis. 3(3), 227-232.

CoE, 1992. Flavouring substances and natural sources of flavourings. 4th Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg.

Colley, J., Gaunt, I.F., Lansdown, A.B.G., Grasso, P., Gangolli, S.D., 1969. Acute and short-term toxicity of diacetyl in rats. Food Cosmet. Toxicol. 7, 571-580.

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard - a decision tree approach. Food Cosmet. Toxicol. 16(3), 255-276.

Dawson, J., Hullin, R.P., 1954. Metabolism of acetoin. 1. The formation and utilization of acetoin and butane-2:3-diol in the decerebrated cat. 2. Metabolic conversions of acetoin, pyruvate and acetate by rabbit-kidney tissue despersions. Biochem. J. 57, 177-185.

DiVincenzo, G.D., Kaplan, C.J., Dedinas, J., 1976. Characterization of the metabolites of methyl n-butyl ketone, methyl iso-butyl ketone, and methyl ethyl ketone in guinea pig serum and their clearance. Toxicol. Appl. Pharmacol. 36, 511-522.

Dorado, L., Montoya, M.R., Rodriguez Mellado, J.M., 1992. A contribution to the study of the structure-mutagenicity relationship for alpha-dicarbonyl compounds using the Ames test. Mutat. Res. 269(2), 301-306.

Eastman Kodak Company, 1992a. Initial submission: The basic toxicity of 2,4-pentanedione with cover letter dated 093092. EPA Doc 88-920008917, microfiche no. 0TS0570692. December 19, 1979. Unpublished report submitted by EFFA to SCF.

EC, 1996a. Regulation No 2232/96 of the European Parliament and of the Council of 28 October 1996. Official Journal of the European Communities 23.11.1996, L 299, 1-4.

EC, 1999a. Commission Decision 1999/217/EC of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs. Official Journal of the European Communities 27.3.1999, L 84, 1-137.

EC, 2000a. Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. Official Journal of the European Communities 19.7.2000, L 180, 8-16.

EC, 2002b. Commission Regulation No 622/2002 of 11 April 2002 establishing deadlines for the submission of information for the evaluation of chemically defined flavouring substances used in or on foodstuffs. Official Journal of the European Communities 12.4.2002, L 95, 10-11.

EC, 2009a. Commission Decision 2009/163/EC of 26 February 2009 amending Decision 1999/217/EC as regards the Register of flavouring substances used in or on foodstuffs. Official Journal of the European Union 27.2.2009, L 55, 41.

EFFA, 2002i. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.

EFFA, 2003e. Submission 2002-4. Flavouring group evaluation of six flavouring substances (candidate chemicals) of the chemical group 10 (Annex I of 1565/2000/EC), structurally related to aliphatic acyclic and alicyclic alpha diketones and related alpha-hydroxyketones [FAO/WHO JECFA 42/51] used as flavouring substances. 30 October 2002. SCOOP/FLAV/8.17.

EFFA, 2003f. Submission 2002-4. Flavouring group evaluation of six flavouring substances (candidate chemicals) of the chemical group 10 (Annex I of 1565/2000/EC), structurally related to aliphatic acyclic and alicyclic alpha diketones and related alpha-hydroxyketones [FAO/WHO JECFA 42/51] used as flavouring substances. 30 October 2002. SCOOP/FLAV/8.17. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to SCF.

EFFA, 2004e. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.

EFFA, 2004x. Submission of 2002-4 Addendum. Supplement of one flavouring substance (candidate chemicals) to the flavouring group evaluation of the chemical group 10 (Annex I of 1565/2000/EC) structurally related to aliphatic acyclic and alicyclic alpha-diketones and related alpha-hydroxyketones [FAO/WHO JECFA 42/51] used as flavouring substances. 31 March 2004. FLAVIS/8.67. Unpublished report submitted by EFFA to FLAVIS Secretariat.

EFFA, 2007a. E-mail from Jan Demyttenaere, EFFA to Flavis Secretariat, National Foodinstitute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages FLAVIS/8.70.

EFFA 2007l. Submission 2007-10. Safety evaluation of aliphatic acyclic and alicyclic alpha-diketones and related alpha-hydroketones used as flavouring agents (S08-J18). Submission 2007_10_EFSA S08-J18. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.103

EFFA, 2010a. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.

EFSA, 2004a. Minutes of the 7th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Brussels, 28 September 2004. [Online]. Available: http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/afc_minutes_07_en1.pdf?ssbinary=true

EFSA, 2005b. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 10: Aliphatic primary and secondary saturated and unsaturated alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones from chemical groups 9, 13 and 30 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 28 October 2005. EFSA-Q-2003-153a.

EFSA, 2009x. Opinion of the Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids on a request from the Commission related to Flavouring Group Evaluation 213: alpha,beta-Unsaturated alicyclic ketones and precursors from chemical subgroup 2.7 of FGE.19 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 27 November 2008. EFSA-Q-2008-768.

Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. [Online]. Available: http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1090,30070682,1090_33076576&_dad=portal&_schema=PORTAL, Population and social conditions, Population, Demography, Main demographic indicators, Total population. December 2008.

FDA (Food and Drug Administration), 1973. Teratologic evaluation of FDA 71-73 (Starter Distillate, Hansen). Food and Drug Research Labs., Inc. Morgareidge K. Lab. no. 1573p. FDABF-GRAS-152. August 20, 1973. Unpublished report submitted by EFFA to SCF.

FDA (Food and Drug Administration), 1974. Mutagenic evaluation of compound FDA 71-73 (starter distillate). Liotton Bionetics, Inc. Brusick, D. FDABF-GRAS-275. Unpublished report submitted by EFFA to SCF.

Flavour Industry, 2005a. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-11.

Flavour Industry, 2005b. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-11.

Frantz, S.W., Ballantyne, B., Leung, H.-W., 1998. Acute intravenous and inhalation pharmacokinetics of 2,4-pentanedione in the Fischer 344 rat. Toxicol. Ind. Health 14(3), 413-428.

Gabriel, M.A., Jabara, H., Al-Khalidi, U.A.S., 1971. Metabolism of acetoin in mammalian liver slices and extracts. Biochem. J., 124, 793-800.

Gabriel, M.A., Ilbawi, M., Al-Khalidi, U.A.S., 1972. The oxidation of acetoin to CO_2 in intact animals and in liver mince preparation. Comp. Biochem. Physiol. 41B, 493-502.

Garst, J., Stapleton, P., Johnston, J., 1983. Mutagenicity of alpha-hydroxy ketones may involve superoxide anion radical. Oxy Radicals and Their Scavenger Systems 2, 125-130.

Gaunt, I.F., Brantom, P.G., Kiss, I.S., Grasso, P., Gangolli, S.D., 1972b. Short-term toxicity of acetoin (acetylmethylcarbinol) in rats. Food Cosmet. Toxicol. 10, 131-141.

Gava, C., Perazzolo, M., Zentilin, L., Levis, A.G., Corain, B., Bombi, G.G., Palumbo, M., Zatta, P., 1989. Genotoxic potentiality and DNA-binding properties of acetylacetone, maltol, and their aluminum(III) and chromium(III) neutral complexes. Toxicol. Environ. Chem. 22(1-4), 149-157.

Gordon, A.J., Ford, R.A., 1972. The chemist's companion. A handbook of practical data, techniques, and references. John Wiley & Sons, New York, pp. 49-51.

Guentert, 2004. Degradation of diacetyl trimer. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Guzzie, P.J., Morabit, E.R., 1985. 2,4-pentanedione: Salmonella/microsome (Ames) bacterial mutagenicity assay. Project report 48-140. EPA Doc FYI-OTS-0286-0434, microfiche no. 0TS0000434-0. December 2, 1985. Unpublished report submitted by EFFA to SCF.

Guzzie, P.J., Morabit, E.R., 1986. 2,4-pentanedione: In vivo mouse micronucleus study. Project report 49-124. EPA Doc 89-870000070, microfiche no. OTS0510542-1. November 21, 1986. Unpublished report submitted by EFFA to SCF.

Heymann, E., 1980. Carboxylesterases and amidases. In: Jakoby, W.B. (Ed.). Enzymatic basis of detoxication. 2nd Ed. Academic Press, New York, pp. 291-323.

IOFI, 1995. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995.

Iwata, T., Kokuba, S., Ariga, F., Hiramatsu, Y., Nose, T., Aoyama, T., 1984. [Mutagenicity of lenampicillin hydrochloride (KBT-1585)B and its metabolites]. Chemotherapy. 32(8), 153-159. (In Japanese)

Järnefelt, J., 1955. Studies on the enzymatic synthesis and breakdown of acetoin in the animal organism. Ann. Acad. Sci. Fennicae Serie A V. Med. Anthropol. 57, 7-78.

JECFA, 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.

JECFA, 1996a. Toxicological evaluation of certain food additives. The forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA, 1997a. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

JECFA, 1998b. Compendium of food additive specifications. Addendum 6. Joint FAO/WHO Expert Committee of Food Additives 51st session. Geneva, 9-18 June 1998. FAO Food and Nutrition paper 52 Add. 6.

JECFA, 1999a. Safety evaluation of certain food additives. The fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food Additives Series: 42. IPCS, WHO, Geneva.

JECFA, 1999b. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

JECFA, 2000a. Evaluation of certain food additives. Fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 9-18 June 1998. WHO Technical Report Series, no. 891. Geneva.

JECFA, 2000d. Compendium of food additive specifications. Addendum 8. Joint FAO/WHO Expert Committee of Food Additives. 55th meeting. Geneva, 6-15 June 2000. FAO Food and Nutrition paper 52 Add. 8.

JECFA, 2001c. Compendium of food additive specifications. Addendum 9. Joint FAO/WHO Expert Committee of Food Additives 57th session. Rome, 5-14 June 2001. FAO Food and Nutrition paper 52 Add. 9.

JECFA, 2003b. Compendium of food additive specifications. Addendum 11. Joint FAO/WHO Expert Committee of Food Additives 61st session. Rome, 10-19 June 2003. FAO Food and Nutrition paper 52 Add. 11.

Jenner, P.M., Hagan, E.C., Taylor, J.M., Cook, E.L., Fitzhugh, O.G., 1964. Food flavorings and compounds of related structure. I. Acute oral toxicity. Food Cosmet. Toxicol. 2, 327-343.

Juni, E., Heym, G.A., 1956. A cyclic pathway for the bacterial dissimilation of 2,3-butanediol, acetylmethylcarbinol, and diacetyl. J. Bacteriol. 71, 425-432.

Kato, F., Araki, A., Nozaki, K., Matsushima, T., 1989. Mutagenicity of aldehydes and diketones. Mutat. Res. 216, 366-367.

Kawano, T.,1959. On the relation of acetoin with pantothenic acid. Fukuoka Igaku Zasshi. 50, 2939-2953.

Kim, S.B., Hayase, F., Kato, H., 1987b. Desmutagenic effect of alpha-dicarbonyl and alpha-hydroxycarbonyl compounds against mutagenic heterocyclic amines. Mutat. Res. 177, 9-15.

Kopf, R., Loeser, A., Meyer, G., 1950. Untersuchungen über die Pharmakologie und Toxikologie mehrwertiger Alkohole (1,3-butylenglykol). Arch. Exp. Pathol. Pharmakol. 210, 346-360. (In German)

Marnett, L.J., Hurd, H.K., Hollstein, M.C., Levin, D.E., Esterbauer, H., Ames, B.N., 1985a. Naturally-occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. Mutat. Res. 148, 25-34.

Matsui, S., Yamamoto, R., Yamada, H., 1989. The Bacillus Subtilis/Microsome rec-assay for the detection of DNA damaging substances which may occur in chlorinated and ozonated waters. Water Sci. Technol. 21, 875-887.

Montgomery, J.A., David, F., Garneau, M., Brunengraber, H., 1993. Metabolism of 2,3-butanediol stereoisomers in the perfused rat liver. J Biol. Chem. 268(27), 20185-20190.

Moreno, O.M., 1977d. Acute oral toxicity in rats. Dermal toxicity in rabbits. Acetyl butyryl, project no. MB 77-1744, August 18, 1977. Acetyl methyl carbinol, project no. MB 77-1691, June 20, 1977. Acetyl propionyl, MB 76-1445, January 25, 1997. MB Research Laboratories, Inc. Unpublished data submitted by EFFA to SCF.

Moreno, O.M., 1979a. Acute oral toxicity in rats. Acute dermal toxicity in rabbits. Acetyl valeryl. MB Research Laboratories, Inc. Project no. MB 78-3418. Date 3/22/79. Upublished data submitted by EFFA to SCF.

Myers, R.C., Carpenter, C.P., Cox, E.F., 1977a. Initial submission: Silane coupling agent: Range finding toxicity studies with cover letter dated 090892. Carnegie-Mellon Institute. Kuryla, W.C. September 8, 1992. EPA Doc 88-920009321, microfiche no. 0TS0571073. Unpublished report submitted by EFFA to SCF.

Myers, R.C., Slesinski, R.S., Frank, F.R., 1985. Initial submission: 2,4-pentanedione: Acute toxicity and primary irritancy studies /final report) with cover letter dated 031892. Bushy Run Research CTR. Kuryla, W.C. Date 4/10/92. EPA Doc 88-920001502, microfiche no. OTS0536178. Unpublished data submitted by EFFA to SCF.

NTP, 1994c. Bone Marrow Micronucleus study (2,3-butanedione). Study no. A44706; http://ntp.niehs.nih.gov/*

Ono, Y., Somiya, I., Kawamura, M., 1991. The evaluation of genotoxicity using DNA repairing test for chemicals produced in chlorination and ozonation processes. Water Sci. Technol. 23, 329-338.

Otsuka, M., Mine, T., Ohuchi, K., Ohmori, S., 1996. A detoxication route for acetaldehyde: Metabolism of diacetyl, acetoin, and 2,3-butanediol in liver homogenate and prefused liver of rats. J. Biochem. 119, 246-251.

Otsuka, M., Harada, N., Itabashi, T., Ohmori, S., 1999. Blood and urinary levels of ethanol, acetaldehyde, and C4 compounds such as diacetyl, acetoin, and 2,3-butanediol in normal male students after ethanol ingestion. Alcohol 17(2), 119-124.

Pellmont, B., 1969a. Studies with rats and mice on substance no. R01-3801. Toxikologisches Labor 256, Bau 69. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Posternak, N.M., Linder, A., Vodoz, C.A., 1969. Summaries of toxicological data. Toxicological tests on flavouring matters. Food Cosmet. Toxicol. 7, 405-407.

San, R.H.C., Klug, M.L., 1993. Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test). 4-Hydroxy-4-methyl-2-pentanone. Microbiological Associates, Inc. Study no. C341.501017. January 28, 1993. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Sasaki, 2006. 3-Methyl-2,4-nonedione: Reverse mutation test "Ames Test" with *S. typhimurium* and *E.coli*. Priviate communication to Research Institute Fragrance Manufacturers (RIFM). Unpublished report submitted by EFFA to FLAVIS Secretariat.

SCF, 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.

SCF, 1999a. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Shane, B.S., Troxclair, A.M., McMillin, D.J., Henry, C.B., 1988. Comparative mutagenicity of nine brands of coffee to *Salmonella typhimurium* TA100, TA102, and TA104. Environ. Mol. Mutag. 11, 195-206

Slesinski, R.S., 1986. 2,4-pentanedione: In vitro genotoxicity studies; CHO/HGPRT gene mutation test; sister chromatid exchange assay (final report) with cover letter dated 013192. Union Carbide Chem. & Plas. Co. EPA Doc 86-92000782, microfiche no. OTS0535115. January 14, 1986. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Smyth Jr., H.F., 1941. Toxicologic test preformed with 2,4-pentanedione with cover letter dated 053086. Union Carbide Corp. Heywood, D.L. EPA Doc 89-8600013, microfiche no. OTSO510542. June 5, 1986. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Smyth Jr., H.F., 1946a. Letter from Union Carbide Corp. to USEPA regarding toxicology studies of diacetone alcohol, with attachments dated 08/25/95. Diaceton alcohol. Union Carbide Corp. EPA Doc 86950000301, microfiche no. 0TS0557741. September 14, 1995. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Smyth Jr., H.F., 1946b. Letter from Union Carbide Corp. to USEPA regarding toxicology studies of diacetone alcohol, with attachments dated 08/25/95. Diaceton alcohol. Union Carbide Corp. EPA Doc 86950000301, microfiche no. 0TS0557741. September 14, 1995. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Stien, J., 2005b. Mutagenicity study of diacetyl-trimer in the *Salmonella typhimurium* reverse mutation assay (in vitro). LPT Report no. 18432/8/04. Laboratory of Pharmacology and Toxicology KG, Hamburg Germany. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Suwa, Y., Nagao, M., Kosugi, A., Sugimura, T., 1982. Sulfite suppresses the mutagenic property of coffee. Mutat. Res. 102, 383-391.

TNO, 2000. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

Veech, R.L., Gitomer, W.L., Casazza, J.P., 1987. Metabolic pathways leading to diol formation. Genet. Alcohol..241, 185-199.

Vergnes, J.S., Kubena, M.F., 1994a. 2,4-pentanedione: Bone marrow micronucleus test in mice, with cover letter dated 11/14/94. Union Carbide Corp. EPA Doc 86950000030, microfiche no. OTSO557543. October 19, 1994. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Vergnes, J.S., Kubena, M.F., 1994b. 2,4-pentanedione: Bone marrow micronucleus test in rats, with letter dated 12/20/94. Union Carbide Corp. EPA Doc 86950000061, microfiche no. OTS0557574. December 14, 1994. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Westerfeld, W.W., Berg, R.L., 1943. Observations on the metabolism of acetoin. J. Biol. Chem. 148(3), 523-528.

Whittaker, P., Clarke, J.J., San, R.H.C., Begley, T.H., Dunkel, V.C., 2008. Evaluation of the butter flavouring chemical diacetyl and a fluorochemical paper additive for mutgenicity and toxicity using the mammalian cell gene mutation assay in L5178Y mouse lymphoma cells. Food Chem. Toxicol. 46, 2928-2933.

Zimmermann, F.K., Mohr A., 1992. Formaldehyde, glyoxal, urethane, methyl carbamate, 2,3-butanedione, 2,3-hexanedione, ethyl actylate, dibromoacetonitrile, 2-hydroxypropionitrile induce chromosome loss in saccharomyces cerevisiae. Mutat. Res. 270, 151-166.

Zimmermann, F.K., Mayer, V.W., Scheel, I., Resnick, M.A., 1985a. Acetone, methyl ethyl ketone, ethyl acetate, acetonitrile and other polar aprotic solvents are strong inducers of aneuploidy in Saccharomyces cerevisiae. Mutat. Res. 149, 339-351.

ABBREVIATIONS

ADI	Acceptable Daily Intake
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
ID	Identity
IOFI	International Organization of the Flavour Industry
IP	Intrapertoneal
IR	Infrared spectroscopy
IV	Intravenous
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50%; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate, reduced form
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food

SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
UDP	Uridine DiPhosphate
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation